

Studies on the biological balance
between thromboxanes and
prostacyclins in relation to the
platelet-vessel wall interaction

Kaj Anker Jørgensen

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Denne afhandling er i forbindelse med omstående tidligere publicerede afhandlinger af det lægevidenskabelige fakultet ved Odense universitet antaget til offentlig at forsvares for den medicinske doktorgrad.

Den 3. november 1981

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The present survey is based upon the following papers

They are referred to in the text with roman numerals.

- I. *Platelets and Atherosclerosis.*
Kaj Anker Jørgensen and Jørn Dyerberg
Dan. Med. Bull. 1980, 27, 253-259.
- II. *Platelets and Renal Disease.*
Kaj Anker Jørgensen
Dan. Med. Bull. 1981, 28, 116-122.
- III. *Platelets and Platelet Function in Patients with Chronic Uremia on Maintenance Hemodialysis.*
Kaj Anker Jørgensen and Steen Ingeberg
Nephron 1979, 23, 233-236.
- IV. *Prostacyclin (PGI_2) and the Effect of Phosphodiesterase Inhibitors on Platelet Aggregation.*
Kaj Anker Jørgensen, Jørn Dyerberg and Erik Stoffersen
Pharmacol. Res. Commun. 1979, 11, 605-615.
- V. *A Biphasic Effect of Prostacyclin (PGI_2) on Platelet Aggregation.*
Kaj Anker Jørgensen, Jørn Dyerberg and Erik Stoffersen
Thromb. Res. 1980, 19, 877-881.
- VI. *Familial Deficiency of Prostacyclin Production Stimulating Factor in the Hemolytic Uremic Syndrome of Childhood.*
Kaj Anker Jørgensen and Robert Smith Pedersen
Thromb. Res. 1981, 21, 311-315.
- VII. *Hydrocortisone, Platelet Aggregation and Platelet Prostaglandin Metabolism.*
Kaj Anker Jørgensen and Erik Stoffersen
Scand. J. Haemat. 1980, 25, 445-447.
- VIII. *On the Inhibitory Effect of Albumin on Platelet Aggregation.*
Kaj Anker Jørgensen and Erik Stoffersen
Thromb. Res. 1980, 17, 13-18.
- IX. *Acetylsalicylic acid, Bleeding Time and Age.*
Kaj Anker Jørgensen, Jørn Dyerberg, Anders Schou Olesen and Erik Stoffersen
Thromb. Res. 1980, 19, 799-805.

X. *Acetylsalicylic acid and Bleeding Time in Juvenile Diabetes Mellitus.*

Kaj Anker Jørgensen, Hans Torben
Mourits-Andersen, Jørn Ditzel and Jørn
Dyerberg

Thromb. Res. 1980, 20, 611-615.

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ABBREVIATIONS

AA:	arachidonic acid.
ADP:	adenosine diphosphate.
ADP _{min} :	minimal ADP concentration (μM) necessary to induce a well defined secondary wave of platelet aggregation.
AMIS:	aspirin myocardial infarction study research group.
ASA:	acetylsalicylic acid.
BT:	bleeding time, suffix indicates ASA mg/kg ingested $1\frac{1}{2}$ -2 hours before determination.
CAM:	calf aortic microsomes.
cAMP:	cyclic adenosine monophosphate.
CP/CPK:	creatin phosphate/creatin phosphokinase.
EFA:	essential fatty acids.
EPA:	eicosapentaenoic acid.
GCS:	glucocorticosteroids.
HbA _{1c} :	hemoglobin A _{1c}
HETE:	12-hydroxy-eicosatetraenoic acid.
HHT:	12-hydroxy-heptadecatrienoic acid.
15-HPAA:	15-hydroperoxy-arachidonic acid.
HPETE:	12-hydroperoxy-eicosatetraenoic acid.
I _a :	$= (\text{BT}_{25} - \text{BT}_0) / \text{BT}_{25}$.
LASS:	labil aggregation stimulating substance.
MDA:	malondialdehyde.
m-RNA:	messenger-ribonucleic acid.
PG:	prostaglandin.
PGI ₂ :	prostacyclin (PGX).
PPP:	platelet poor plasma.
PRP:	platelet rich plasma.
PSF:	prostacyclin production stimulating factor.
RAT:	a piece of rat aortic tissue.
RCS:	rabbit aorta contracting substance.
TCM:	tranylcypromine.
TRIS:	tris (hydroxymethyl) nitromethane.
TXA ₂ :	thromboxane A ₂ .
TXB ₂ :	thromboxane B ₂ (PDH).

PREFACE

The studies on which this survey is based were carried out at the Department of Clinical Chemistry, section North, Aalborg Hospital, where I was employed 1978-1981. I owe a debt of gratitude to the head of the department, Jørn Dyerberg, for encouragement, valuable guidance, neverfailing interest, and excellent working conditions. I also express my sincere gratitude to med. techn. Erik Stoffersen for the outstanding collaboration, which has been the fundament of this study.

The earliest studies were carried out during my employment at the Department of Medicine and Nephrology, Aalborg Hospital, 1976-1978. The friendly support of the physician-in-chief, Arne W. S. Sørensen is sincerely acknowledged. My sincere thanks are also due to my other co-authors for generous assistance and fruitful discussions. My thanks also go to secretary Jane Holst for type-writing the manuscript. Acknowledgement is also given to »Thrombosis Research«, »Pharmacological Research Communications« and »Scandinavian Journal of Haematology« for kind permission to use figures and tables, which have been published previously. The help of the personnel at The Animal House, Institute of Pathology, Aalborg Hospital, is also sincerely acknowledged.

I am grateful to my wife, Elly, for support and help throughout this study.

This survey was finished in November 1980 and submitted to the Faculty of Medicine, Odense University, on January 9th, 1981, and accepted as a doctorate thesis to be publicly defended.

INTRODUCTION

Vessel injury in mammals is immediately followed by accumulation of platelets at the point of injury and stabilization of the clump of platelets by formation of a coagulum of protein (Bizzorero 1982, Zucker 1947, Jørgensen and Borchgrevink 1963, Hovig et al 1967). Upon disruption of the endothelium platelets adhere to elements of vessel wall or perivascular tissue with an astonishing speed (Wright and Minot 1917, Bournameaux and Roskam 1959, Hugues 1962, Baumgartner et al 1971, Baumgartner 1972, Baumgartner 1973, Baumgartner 1974, Born 1977). Platelets are also important for the integrity of the normal vascular bed (Kitchens and Weiss 1975).

The aggregation of platelets is stimulated by substances such as collagen, adenosine diphosphate (ADP), epinephrine, arachidonic acid, and thrombin (Apits 1939, Pinninger and Prunty 1946, Bigelow 1954, Gaarder et al 1961, Born 1962, Zucker and Borrelli 1962, O'Brien 1963, Mitchell and Sharp 1964, Hugues and Lapier 1964, Honour and Mitchell 1967, Silver et al 1973). When small amounts of ADP are added to platelets in plasma, they undergo a rapid isovolumic change in shape from disks to spiny spheres and aggregate reversibly (first phase aggregation) while larger amounts of ADP induce irreversible (second phase) aggregation (Hovig 1962, Born and Cross 1963, O'Brien 1965, Constantine 1966, MacMillan 1966, White 1968). The second phase but not the first phase of ADP induced aggregation or thrombin induced aggregation is inhibited by acetylsalicylic acid (Zucker and Peterson 1968, Packham et al 1977).

The release reaction of platelets is a secretory process whereby substances stored in specific granules are extruded during second phase aggregation (Grette 1962, Zucker and Peterson 1967, Mills et al 1968, Holmsen et al 1969, Stormorken 1969, Day and Holmsen 1971, Charo et al 1977). Among these substances are ADP, serotonin, platelet factor 4, factors which increase vessel permeability, a mitogenic factor for smooth muscle cells, and platelet factor 3 which activates the coagulation cascade consolidating the aggregated platelets with fibrin (Haslam 1964, Packham et al 1968, Marcus 1969, Nachman et al 1972, Joist et al 1974, Ross et al 1974, Walsh 1974, Busch et al 1976).

Platelets contain an actomyosin-like protein and many platelet activities like change in shape, aggregation, release reaction, and clot retraction have been attributed to this contractile protein (Lüscher and Betex-Galland 1972). Platelet aggregation and secretion is believed to be mediated by the concentration of free calcium in the cytosol and its interaction with cyclic adenosine monophosphate (cAMP) and protein kinases (Salzman and Neri 1969, Rasmussen 1970, Salzman 1972, Durham 1974, Berridge 1975, Massini and Lüscher 1976, Detwiler et al 1978, Massini et al 1978, Gerrard et al 1978, Haslam et al 1978). Increase in free calcium concentration in the cytosol can be achieved from intracellular calcium deposits resulting in aggregation and cAMP stimulates uptake of calcium by platelet membrane vesicles (Feinman and Detwiler 1974, White et al 1974, Käser-Glanzmann et al 1977, Pickett et al 1977, Rittenhouse-Simmons et al 1978). Increase in intracellular cAMP level inhibits platelet aggregation (Ardlie et al 1967, Mannucci et al 1974, Salzman et al 1974).

Besides being important in maintaining the integrity of the vascular bed platelets have been increasingly implicated as pathogenetic important factors in several common human diseases. Studies on factors governing platelet functions are therefore of great interest. This survey deals with the balance between two very potent labile endogenous substances, the proaggregatory thromboxane and the antiaggregatory prostacyclin, implemented on the possible pathophysiological role a shift in this balance might have in various clinical conditions.

Chapter I

MATERIALS AND METHODS

The following section gives a brief description of standard methodological procedures. Description of methods used under special experimental conditions will be given in the appropriate section.

Venous blood was collected from a cubital vein after minimal or no stasis into sodium citrate. (0.11 M), one part of citrate to nine parts of blood. When acetylated platelets were to be used, the blood was

collected from a healthy young male volunteer who had taken 2000 mg of acetylsalicylic acid (ASA) daily for 2 days before sampling whereas normal platelets were harvested from the same volunteer after he had been free of any medication for 3 weeks.

Platelet rich plasma (PRP) was obtained by centrifugation at 200 g for 5 minutes and platelet poor plasma (PPP) at 1500 g for 10 minutes. Washed platelets were prepared by recentrifugation of PRP at 200 g for 5 minutes. The platelet pellet was then transferred to the washing solution while the plasma supernatant was centrifugated again in order to harvest as many platelets as possible. This procedure was then repeated and the final solution adjusted to contain 250×10^9 platelets/l. In early experiments this procedure resulted in a great loss of platelets, especially in normal non-acetylated platelets. In later experiments PGI_2 1 ng/ml was added to the PRP just prior to the washing procedure. The platelets were then used at least one hour after the washing procedure, at which time the effect of PGI_2 had completely disappeared as checked by thrombin induced aggregation.

Platelet aggregation studies were carried out in a Fibromat® (Bie and Berntsen, Copenhagen, Denmark) and recorded as alternation in light transmission at 37° C in plastic cuvettes containing 300 μl stirred magnetically at 800 rpm. Aggregation was expressed as the maximal alteration in light transmission of the platelet suspension upon induction of aggregation relative to the difference between light transmission of a control suspension without platelets and the suspension of unstimulated platelets.

Ivy bleeding times were determined by duplicate measurements on the forearm utilizing the Simplate II bleeding time device (General Diagnostics, New Jersey), the results given to the nearest $\frac{1}{2}$ minute.

Malondialdehyde generation of a platelet suspension was determined as described by Smith et al (1976) after incubation with either 1 mM arachidonic acid or 1.5 U/ml thrombin for 10 minutes at 37° C. The mean of two determinations with appropriate blanks was used as the result. TXB_2 was measured by radioimmunoassay after acidification with formic acid to pH=3.5 and extraction with double volume ethylacetate.

Pieces of aortic tissue weighing approximately 15

mg wet weight were prepared from freshly killed Witstar rats. Human umbilical cords were obtained just after delivery and frozen at $\div 70^{\circ}\text{C}$ until preparation within 3 weeks. Calf aortas were obtained at the local slaughterhouse and prepared within 2 hours. The adventitia and most of the muscular layer was stripped off the calf aortas. Umbilical blood vessels were carefully dissected free of the cords. The endothelial tissue was cut in small pieces, suspended in TRIS-buffer, pH=7.4 and homogenized in a high speed homogenesator (Ultra-Turrax®, Jankel and Kunkel KG, Staufen, Germany). The homogenate was frozen, crushed and then thawed before centrifugation for 10 minutes at 5000 g. The supernate was then centrifugated at 100,000 g for 60 minutes. The pellet was resuspended in TRIS-buffer pH=7.4 to yield a protein concentration of 1.16 mg/ml (Lowry's method), and stored at $\div 70^{\circ}\text{C}$ until use.

Reagents

ADP (Adenosine-5'-diphosphate®) and arachidonic acid (99 %) from Sigma Chemicals, St. Louis, USA. Thrombin (Thrombin reagent Leo, bovine origin) from Leo Pharmaceutical Products, Copenhagen, Denmark. ^3H -TXB₂ from New England Nuclear, Boston, USA. TXB₂, anti-TXB₂ and PGI₂ were kindly supplied by The Wellcome Research Laboratory, Beckenham, England. PGI₂ powder was dissolved to a stock solution of 1 mg/ml in TRIS-buffer pH=9.0 which kept on ice was stable for 8 hours. Working solutions of an appropriate PGI₂ concentration in TRIS-buffer pH=7.4 were prepared on ice 20 seconds before each experiment from the stock solution. Other standards and medical drugs were purchased from normal commercial sources.

Chapter II

PLATELETS AND SOME HUMAN DISEASES

1. Platelets and thrombosis

A platelet aggregate developing at the site of vessel damage is a haemostatic plug, but if bloodflow is prevented it becomes a thrombus. The drawings of Ebert and Schimmelbusch published in 1886 show that they understood the importance of platelets in throm-

bus formation as it has been demonstrated in many studies since (Mustard and Packham 1970, Pavlovsky et al 1971, Baumgartner 1972). Platelets are especially important in the development of arterial thrombosis which can occur independently of the coagulation system (Baumgartner 1973, Harker and Slichter 1974, Baumgartner et al 1976). The role of platelets in thrombosis has also been stressed by studies of platelet survival and studies at autopsies (Harker and Slichter 1972b, Salky and Dugdale 1973, Weiley et al 1974, Hærem 1974, Hudson and McCaughey 1974, Steele et al 1978, Kutti and Weinfeld 1979). However, platelets do not adhere to normal endothelium and quite a substantial endothelial lesion is required to produce a thrombus (French et al 1964, Ashford and Freiman 1967). It therefore seems that in arteries thrombosis is a result of endothelial damage (Spaet and Erichson 1966) which very often will be an atherosclerotic lesion (Chapman 1965, Constatinides 1966).

2. Platelets and atherosclerosis

(I. Jørgensen and Dyerberg)

Von Willebrand swine, thrombocytopenic rabbits and dipyridamole treated baboons are all resistant to experimental atherosclerosis (Harker et al 1976, Moore et al 1976, Fuster et al 1978). Platelets may therefore play an important role in atherogenesis. Stimulated platelets release a mitogenic factor, which strongly stimulates proliferation of muscle and connective tissue cells (Ross and Vogel 1978). Since most theories on atherogenesis include such a proliferation in the intimal cells, the platelet mitogenic factor could play a role in the development of the primary myointimal lesion. The role of platelets in atherosclerosis has newly been reviewed (I. Jørgensen and Dyerberg).

3. Platelets and renal disease

(II. Jørgensen, III. Jørgensen and Ingeberg)

Evidence from both experimental and human glomerulonephritis suggests that platelets may play a role in the deposition of immune complexes and inflammation associated with some types of glomerulonephritis (Wardle 1972, Clark et al 1976, Cameron 1977). Increased platelet turnover, decreased intraplatelet and increased plasma serotonin have been demonstrated in types of

glomerulonephritis which usually progress to renal failure (Cameron 1977, Parbtani and Cameron 1979).

The pathogenesis of the haemolytic uraemic syndrome is still unknown (Kaplan and Drummond 1978, Jørgensen 1978). Platelets do, however, seem to play an important role in the early stages of the diseases (George et al 1974, Riella et al 1976).

The occurrence of haemorrhage as a complication of uremia has been recognized for many years. It has been ascribed to capillary fragility, coagulation and fibrinolytic defects. However, recently many authors have ascribed the bleeding to a qualitative platelet defect. The author has studied the ADP ($4\mu\text{M}$), ristocetin (1.55 mg/ml), epinephrine (0.09 mg/ml), fibrinogen (0.27 mg/ml), and collagen ($18\mu\text{g/ml}$) induced aggregation of PRP from 20 uraemic patients on maintenance haemodialysis who had not taken any drugs known to affect platelet function for a period of 10 days. There was found no difference between these patients and 26 healthy volunteers (III. Jørgensen and Ingeberg). The platelet hypoaggregability described by other investigators is therefore probably due to methodological differences or to the medication of the patients (Lindsay et al 1975, Nenci et al 1979). The abnormalities thus demonstrated must be small and cannot account for serious bleeding alone. However, 2 of the 20 uraemic patients had greatly prolonged bleeding times in agreement with many other studies. It may therefore be that these laboratory tests do not reflect the »in vivo« situation. The role of platelets in these renal diseases has been reviewed recently (II. Jørgensen).

4. Platelets and diabetes mellitus

In 1964 Powell and Field reported an unexpectedly low incidence of retinopathy in diabetics with rheumatoid arthritis and suggested that this may be due to the use of acetylsalicylic acid in rheumatoid arthritis. Although this finding has not been confirmed numerous reports have appeared documenting increased platelet adhesiveness, platelet hyperaggregability, shortened platelet survival, increased plasma beta-thromboglobulin, and platelet shape change abnormalities in patients with diabetes of substantial duration and clinical evidence of angiopathy (Mayne et al 1970, Heath et al 1971, Kwaan et al 1972, Bensoussan et al 1975, Breddin et al 1976,

Mustard and Packham 1977, Collier et al 1978, Preston et al 1978, Porta et al 1980, Borsey et al 1980). Reports on platelet abnormalities early in diabetes are very few (Sagel et al 1975). It is therefore uncertain if platelet abnormalities are a result of vascular damage or if they are involved in the genesis of diabetic angiopathy.

Thromboxane and prostacyclin have lately been implicated in atherosclerosis, renal disease and diabetes mellitus. These implications will be dealt with in the following chapters.

Chapter III

THROMBOXANE AND PROSTACYCLIN

1. Prostaglandins

The first observations of an effect of prostaglandins are credited to Kurzrok and Lieb (1930), but it was von Euler (1936) who named the blood pressure lowering compound in extracts of prostate and seminal vesicles »prostaglandin«. Bergström et al (1963) showed that prostaglandin was not a single substance and published the structure of two of them (PGE_1 and $\text{PGF}_{1\alpha}$). Shortly afterwards it was shown that arachidonic acid is the precursor of PGE_2 and $\text{PGF}_{2\alpha}$ (van Dorp et al 1964, Bergström et al 1964). Studies on the origin of the oxygen atoms lead to the postulate of an endoperoxide intermediate in prostaglandin synthesis (Samuelsson 1965). These endoperoxide intermediates were subsequently isolated (Hamberg and Samuelsson 1973, Nugteren and Hazelhof 1973) and the conversion of endoperoxide to PGE_2 and $\text{PGF}_{2\alpha}$ characterized (Wlodawer and Samuelsson 1973).

The first step in the biosynthesis of prostaglandins from arachidonic acid is a cyclooxygenation by incorporation of two oxygen molecules, one between C 9 and C 11 the other at C 15, concomitant with cyclization of the carbon chain at C 8 and C 12 (Samuelsson 1972, fig. 1). The unstable endoperoxide produced is called PGG_2 . The hydroperoxy group at C 15 is then converted to a hydroxy group yielding the endoperoxide termed PGH_2 (Hamberg and Samuelsson 1973, Nugteren and Hazelhof 1973, Hamberg et al 1974a). Both activities seem to be present in the same enzyme protein, the prostaglandin endoperoxide synthetase which is membrane bound and associated with

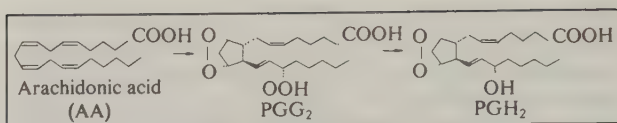


Figure 1. *The prostaglandin endoperoxide synthetase.*

the microsomes (Miyamoto et al 1976, Helmer et al 1976, van der Ouderaa et al 1977, van Dorp et al 1978). The enzyme is acetylated by acetylsalicylic acid (ASA) which inhibits the cyclooxygenase activity of the enzyme (Roth and Majerus 1975, Roth et al 1975, Vane 1976, Rome and Lands 1976, van Dorp et al 1978).

PGH₂ can decompose both spontaneously and enzymatically to the classical prostaglandins PGF_{2 α} , PGE₂ and PGD₂ (van Dorp et al 1978, fig. 2). Other fatty acids can also be used by the prostaglandin synthetase enzyme complex. Δ 8, 11, 14 eicosatrienoic acid yields prostaglandins of the 1 series (PGE₁, PGD₁, etc.) while Δ 5, 8, 11, 14, 17 eicosapentaenoic acid yields prostaglandins of the 3 series (PGE₃, PGD₃, etc.) (Samuelsson 1978).

A great number of different prostaglandins with a variety of pharmacological reactions have emerged from the intensive research in this area. Their exact role in human physiology is far from completely defined, but there is a great deal of evidence showing that they do play a role in many physiological functions (Flack 1973, Markelonis and Garbus 1975, Kenimer et al 1977, Barden 1977, Mathé 1977, Robert 1977, Flamenbaum and Kleinman 1977, Kuehl et al 1977, Blumberg et al 1977, Malik 1978, Wasserman 1979). Since prostaglandins are produced in and have an effect on almost all organs, it is not surprising that platelets produce and are affected by prostaglandins.

2. Stable prostaglandins and platelets

Kloeze (1967, 1969) was the first to show that PGE₁ inhibited platelet aggregation induced by ADP and it was subsequently shown that PGE₁ inhibited the platelet aggregation induced by many different substances (Emmons et al 1967, Shio et al 1970, Sekhar 1970, Kinlough-Ratbone et al 1970). This inhibition is mediated by activating the membrane-bound adenylate cy-

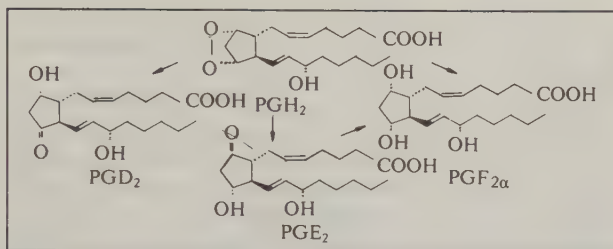


Figure 2. *The spontaneous and enzymatic degradation of the prostaglandin endoperoxide PGH_2 to classical prostaglandins.*

clase, thus increasing the intracellular cAMP level (Salzman 1974, Smith and MacFarlane 1974). PGE_1 stimulates the adenylate cyclase of broken cell preparations from platelets (Marquis et al 1969, Moskowitz et al 1971) and increases the cAMP content of intact platelets (Vigdahl et al 1969, Mills and Smith 1972, Haslam 1973, Mills and MacFarlane 1977). The effect of PGE_1 on both cAMP and aggregation is potentiated by phosphodiesterase inhibitors (Marquis et al 1969, Mills and Smith 1971, Rossi and Levin 1973, Mannucci et al 1974) and prevented by inhibitors of the adenylate cyclase enzyme (Haslam et al 1978, Salzman and MacIntyre 1978).

PGD_2 is an even more powerful platelet aggregation inhibitor and adenylate cyclase stimulator (Smith et al 1974b, Mills and MacFarlane 1974, Nishizawa et al 1975, Whittle et al 1978), although there is great difference in the effect of PGD_2 on platelet aggregation in different species.

$PGF_{2\alpha}$ has no effect on platelet aggregation. PGE_2 usually produces a variable stimulation of the second wave of aggregation of human platelets, but has no effect on the primary wave or on ASA treated platelets (Shio and Ramwell 1972). At very high concentrations PGE_2 can have inhibitory effects on platelet aggregation (Kloeze 1967, Irion and Blombäck 1969). Inhibition of aggregation induced by PGD_2 is prevented by doses of PGE_2 which are equal to or only slightly higher than the doses of PGD_2 (Gryglewski et al 1979).

Since stable prostaglandins are removed rapidly from the circulation they probably have a limited role as circulating hormones, but they probably act locally at

their site of action (Ferreira and Vane 1967, McGiff et al 1969, Piper et al 1970, Hamberg and Samuelsson 1971).

Platelets also produce prostaglandins. In 1970 Smith and Willis demonstrated that human platelets produced small amounts of PGE_2 and $\text{PGF}_{2\alpha}$ upon stimulation with thrombin. It was later demonstrated that platelets also can produce prostaglandins of the '1', '2' and '3' series (Clausen and Srivastava 1972). Platelets also produce PGD_2 during aggregation (Oelz et al 1976a, 1977).

It has been known for some time that ASA inhibits the second wave of ADP, collagen and epinephrine induced aggregation (Gast 1964, O'Brien 1968, Evans et al 1968). The finding that the mechanism of action of ASA is inhibition of prostaglandin synthesis and that ASA inhibits platelet prostaglandin formation indicated that prostaglandin synthesis was involved in platelet function (Vane 1971, Smith and Willis 1971).

Arachidonic acid induces platelet aggregation. Intravenous injection of arachidonic acid into rabbits kills them within 2 minutes by producing platelet aggregates in the small vessels of the lung. ASA has a protective effect in these experiments and injection of corresponding amounts of stable prostaglandins has a much less dramatic effect (Silver et al 1973, Silver et al 1974, Smith and MacFarlane 1974). When platelets are stimulated with radioactive arachidonic acid, only a small fraction of the radioactivity is detected as stable prostaglandins (PGE_2 , PGD_2 and $\text{PGF}_{2\alpha}$) (Hamberg and Samuelsson 1974, Hamberg et al 1974b, Smith et al 1975).

It therefore seemed clear that other metabolites of arachidonic acid than the known stable prostaglandins were involved in the mediation of platelet aggregation.

3. *Thromboxane*

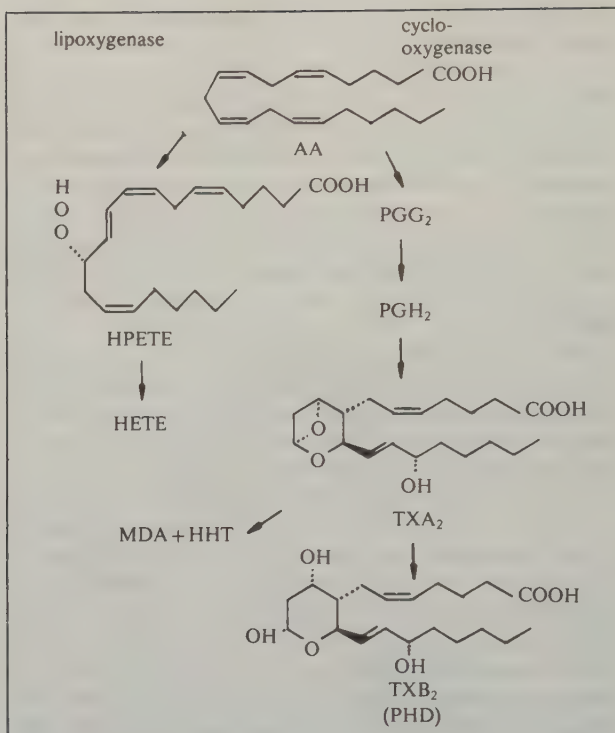
The endoperoxides PGG_2 and PGH_2 were shown to be very potent in inducing rapid and irreversible aggregation of human platelets and it was suggested that they were the mediators of platelet aggregation and release reaction (Hamberg et al 1974a, b, Smith et al 1974a, Malmsten et al 1975). The action of the labile aggregation stimulating substance (LASS) produced from arachidonic acid by sheep vesicular gland preparations

was shown to be due to endoperoxides (Willis et al 1974, Willis 1974a, b).

In 1969 Piper and Vane described a labil rabbit aorta contracting substance (RCS) which was released from lungs during anaphylaxis. It is also released from guinea pig lung following perfusion with arachidonic acid and from human platelets after addition of thrombin. The release was blocked by ASA (Collier 1969, Svensson et al 1975). It is a very unstable substance with a halflife of about 30 seconds at 37°C compared to a halflife of about 5 minutes for endoperoxides.

Hamberg and Samuelsson (1974) discovered that arachidonic acid was transformed by platelets by two main pathways (fig. 3). In one pathway the initial step was catalyzed by a lipooxygenase and a 12-hydroxy-eicosatetraenoic acid (HETE) was formed as the final product. In the second pathway, arachidonic acid was transformed into endoperoxides. These were almost exclusively converted into a 17-carbon 12-hydroxy fatty acid (HHT), malondialdehyde and a hemiacetal derivate, 8-(1-hydroxy-3-oxopropyl)-9-12L-dihydroxy-5, 10-heptadecadieenoic acid, which was provisionally called PHD. Thrombin aggregation of washed platelets is accompanied by release of large amounts of the final products from both pathways of arachidonic acid metabolism (Hamberg et al 1974b).

The significance of the metabolic transformation of PGG_2 into PHD became apparent by the discovery of a biologically very active intermediate in this transformation. This intermediate was trapped by addition of methanol, ethanol or sodium azide to suspensions of human washed platelets incubated 30 seconds with arachidonic acid or PGG_2 . It was very unstable with a halflife of 32 seconds at 37°C. It was very potent in inducing irreversible platelet aggregation and serotonin release. Experiments using $^{18}\text{O}_2$, ($^{14}\text{C}_1$, $^3\text{H}_8$)-arachidonic acid and $\text{CH}_3\text{O}^2\text{H}$ for trapping the intermediate together with the halflife indicated the presence of a bicyclic oxane-oxetane ring system in the intermediate which therefore was named thromboxane A_2 (TXA_2) and PHD was renamed thromboxane B_2 (TXB_2) (Hamberg et al 1975). The structure of TXB_2 has been confirmed by synthesis (Nelson and Jackson 1976, Kelly et al 1976, Schneider and Morge 1976). TXA_2 is identical to at



AA: Arachidonic acid
 TXA₂: Thromboxane A₂
 MDA: Malondialdehyde
 HHT: 12-hydroxy-heptadecatrienoic acid
 HPETE: 12-hydroperoxy-eicosatetraenoic acid
 HETE: 12 hydroxy-eicosatetraenoic acid.

Figure 3. *The metabolism of arachidonic acid in platelets.*

least the major component of RCS (Svensson et al 1975, Hamberg et al 1975, Bunting et al 1976a).

Thromboxane A₂ is much more potent than endoperoxides as a vasoconstrictor and platelet aggregation stimulator, (Needleman et al 1976a, b, Samuelsson 1977, Svensson 1978). Although some observations suggest that endoperoxides can mediate platelet aggregation without TXA₂ formation (Corey et al 1975, Needleman et al 1976a, b, Needleman et al 1977a, Smith et al 1977), it is, however, generally accepted that TXA₂ is the main functionally active compound of the arachidonic path-

way in platelet aggregation (Gorman et al 1977a, Fitzpatrick et al 1978, McMillan et al 1978). The thromboxane synthetase is located in the microsomes (Sun 1977).

The mode of action of endoperoxides and thromboxane in platelets is not known. They decrease the increase in cAMP level brought about by adenylate cyclase stimulators (Claesson and Malmsten 1977, Gorman et al 1977b, Gorman et al 1978). TXA₂, however, does not lower the basal level of cAMP which suggests that its effect on cAMP is by an indirect mechanism.

There is some disagreement upon the relationship between ADP and TXA₂ in platelet aggregation. Claesson and Malmsten (1977) were of the opinion that TXA₂ aggregation was mediated through ADP release. They found that platelet ADP depletion with creatin phosphate/creatin phosphokinase (CP/CPK) inhibited PGG₂ induced platelet aggregation. Malmsten et al (1977) demonstrated a normal ADP, but an inhibited PGG₂ induced aggregation in a patient with Hermansky-Pudlak syndrome despite normal TXA₂ production from arachidonic acid or PGG₂. These patients are deficient in platelet stored ADP. Likewise most mongrel dogs have platelets which aggregate with ADP, but not with arachidonic acid although TXA₂ synthesis is normal (Johnson et al 1979a). These observations seem to indicate that TXA₂ induces secondary platelet aggregation by inducing ADP release (Samuelsson 1977, 1978).

There are, however, many observations indicating that TXA₂ formation plays a dominant role in secondary platelet aggregation. Platelets from cats with Chediak-Higashi syndrome lack platelet stored ADP and serotonin. Arachidonic acid induces aggregation in a cyclooxygenase dependent manner and serotonin induces biphasic aggregation accompanied by TXB₂ formation without detectable ADP secretion in platelets from these cats (Meyers et al 1979). Minkes et al (1979) reported that arachidonic acid induced platelet aggregation independent of ADP release in a patient with storage pool disease. Charo et al (1977) reported that endoperoxides and TXA₂ in low concentrations can induce platelet aggregation in the absence of secretion. Arachidonic acid also induces irreversible aggregation

in thrombin degranulated platelets which lack releasable ADP (Kinlough-Rathbone et al 1976). In contrast to Claesson and Malmsten (1977) Gerrard et al (1977) did not find any inhibition of CP/CPK on PGG₂ induced platelet aggregation. It has therefore been suggested that the initial aggregatory event induced by TXA₂ is a transport of calcium from a bound to a free state in the cytosol (Gerrard et al 1977, Gorman et al 1978).

4. Prostacyclin

In 1976 it was reported that aortic microsomes contained an enzyme which transformed prostaglandin endoperoxides into an unstable substance called PGX which inhibited platelet aggregation and relaxed blood vessels. It was released from fresh vascular rings spontaneously. The release could be stimulated by arachidonic acid, while indomethacin abolished the release. Vascular rings treated with indomethacin could still generate PGX from endoperoxides (Moncada et al 1976a, 1977a, Bunting et al 1976b, Gryglewski et al 1976). PGX does not only inhibit platelet aggregation induced by arachidonic acid, ADP and thrombin, it also reverses the aggregation of irreversibly aggregated platelets (Moncada et al 1976c, Ho et al 1977, Gryglewski et al 1978b). It was proposed that the generation of PGX by intact vessel wall was the biochemical mechanism underlying its unique ability to resist platelet adhesion and aggregation and that PGX formation was of utmost importance for the control of the extent of thrombus formation in vessels (Bunting et al 1977).

Shortly afterwards PGX was renamed prostacyclin and the structure shown to be 9-deoxy-6, 9 α -epoxy- Δ^5 -PGF_{1 α} by comparison of the biological and chemical properties of the synthetic compound and prostacyclin. It was also found that prostacyclin rapidly decomposes to 6-keto-PGF_{1 α} (Johnson et al 1976). It was anonymously given the name PGI₂ (1977). In the search for the structure of prostacyclin, the investigators were helped by earlier reports showing that forestomach homogenates converted arachidonic acid to 6-keto-PGF_{1 α} (Pace-Asciak 1976a, b). 9-deoxy-6, 9 α -epoxy- Δ^5 -PGF_{1 α} had been detected as a minor arachidonate metabolite in forestomach (Pace-Asciak and Wolfe 1971, Pace-Asciak and Nashat 1977). Still other groups

arrived at the same structure by synthesis (Corey et al 1977, Fried and Barton 1977, Johnson et al 1977, Whittaker 1977).

Prostacyclin is 30-40 times more potent than PGE_1 and 10-20 times more potent than PGD_2 as a human platelet antiaggregatory agent (Moncada et al 1976a, Gryglewski et al 1976, Whittle et al 1978). PGI_2 also inhibits platelet adhesion to collagen and components of the subendothelium (Cazenave et al 1979) and it also inhibits platelet aggregation in vivo (Bayer et al 1979, Bourgain 1979).

Prostacyclin production in vessel walls is greatest in the intima and decreases towards the adventitia, while the proaggregatory activity of vessel wall decreases from adventitia towards the endothelium (Moncada et al 1977b, Herman et al 1977, Silberbauer et al 1978a). The PGI_2 production of cultured endothelial cells, fibroblasts and smooth muscle cells has been studied intensively (Weksler et al 1977, Baenziger et al 1977, 1979, Marcus et al 1978, Nordøy et al 1978). It is generated and continuously released in measurable amounts into the arterial circulation from lungs and not, like other prostaglandins, inactivated by passage through lungs. This has led to the concept that PGI_2 is a circulating hormone (Moncada et al 1978a, Gryglewski et al 1978c, Dusting et al 1978, Hensby et al 1979). This concept has, however, been challenged by others (Steer et al 1980).

Prostacyclin decomposes in aqueous solutions and most biological systems to 6-keto- $\text{PGF}_{1\alpha}$ (Johnson et al 1976) (fig. 4). Experiments have shown that PGI_2 can be metabolized to a number of different dihydro and diketo derivatives of $\text{PGF}_{1\alpha}$ (Pace-Asciak et al 1977, Sun and Taylor 1978, Wong et al 1978, 1979). The metabolite 6-keto- PGE_1 has aroused special attention, since it has been claimed to be physiologically equal to and as active as prostacyclin (Lee et al 1979, Quilley et al 1979, 1980), but others have found PGI_2 to be at least 20 times more potent than 6-keto- PGE_1 (Miller et al 1980).

MECHANISM OF ACTION

It is believed that prostacyclin inhibits platelet aggregation by stimulating the enzyme adenylate cyclase. Prostacyclin increases platelet cAMP (Best et al 1977,

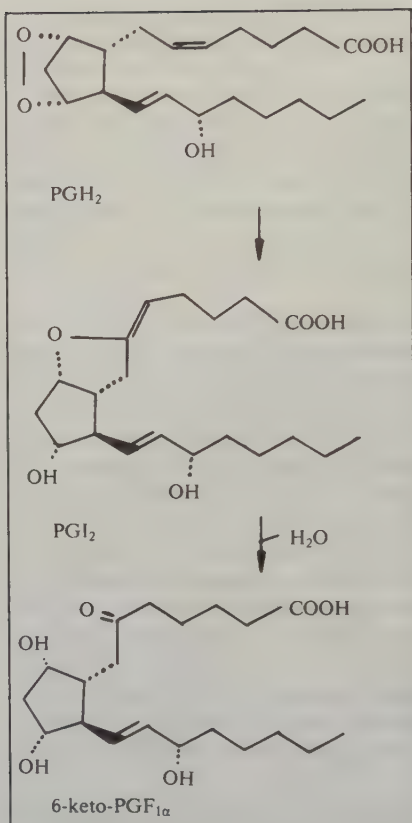


Figure 4. *The synthesis and decomposition of prostacyclin.*

Tateson et al 1977, Gorman et al 1977b, 1978) and its effect on platelets is inhibited by inhibitors of the adenylate cyclase enzyme (Salzman et al 1978). If prostacyclin exerts its platelet inhibitory effects by elevating cAMP, phosphodiesterase inhibitors such as theophylline and dipyridamole would potentiate and prolong the effect of prostacyclin in platelets. If the effect of phosphodiesterase inhibitors is dependent upon prostacyclin in its mechanism of action, it would be unwise to use these drugs in combination with drugs which inhibit PGI₂ production as it is often done in clinical situations. The following studies were therefore

undertaken to demonstrate a relationship between the effect of theophylline and dipyridamole on platelet aggregation and prostacyclin.

THE PRESENT AUTHORS' INVESTIGATIONS

(IV, Jørgensen et al, V, Jørgensen et al)

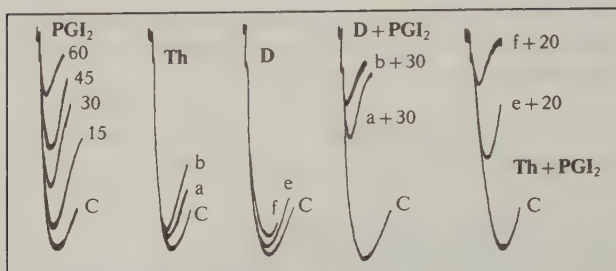
MECHANISM OF ACTION OF PHOSPHODIESTERASE INHIBITORS

(IV, Jørgensen et al)

In the following experiments dipyridamole was primarily administered as pure dipyridamole (Boehringer-Ingelheim, Germany) dissolved in a stock solution of 5 mg/ml in ethanol. In all control experiments the corresponding amount of ethanol was used. All experiments were repeated with Persantin® (Boehringer-Ingelheim, Germany) yielding the same results. Pure theophylline (Ph. Nord. 63) was dissolved in 0.9 % NaCl to a stock solution of 0.2 mg/ml. These agents were added to PRP prior to the washing procedure and to all washing fluids (0.9 % NaCl), because earlier experiments had shown that dipyridamole had platelet stimulating effects when added to washed acetylated platelets (Jørgensen and Stoffersen 1978).

When dipyridamole (5µg/ml) or theophylline (20 µg/ml) was added to PRP, the inhibitory effect on ADP (5 µM) induced aggregation was small and of the same magnitude in normal and acetylated platelets. This very poor effect of dipyridamole in »in vitro« laboratory tests has been demonstrated earlier (Emmons et al 1965, Buchanan and Hirsh 1978).

The experiments shown in fig. 5 and table 1 demonstrate that theophylline and dipyridamole potentiate the effect of prostacyclin on platelet aggregation. The experiments were repeated five times each time demonstrating a potentiating effect. This has also been demonstrated by Whittle et al (1978) and Moncada and Korb (1978b) demonstrated a synergism of the effect of dipyridamole and prostacyclin measured as the weight of collagen strips superfused with rabbit blood. When the rabbits were treated with a high dose of ASA, the effect of dipyridamole greatly decreased demonstrating the requirement of endogenous prostacyclin for the dipyridamole effect.



- a: Theophylline 20 $\mu\text{g/ml}$
b: Theophylline 100 $\mu\text{g/ml}$
e: Dipyridamole 5 $\mu\text{g/ml}$
f: Dipyridamole 25 $\mu\text{g/ml}$
numbers indicate PGI_2 concentration in 10^{-11} g/ml
c: Control ADP aggregation of PRP.

Figure 5. The effect of theophylline (Th), dipyridamole (D), prostacyclin (PGI_2), $\text{D} + \text{PGI}_2$ and $\text{Th} + \text{PGI}_2$ on the ADP (5 μM) induced aggregation of PRP containing acetylated platelets.

Table I. The concentration in 10^{-11} g/ml to which inhibition of platelet aggregation corresponds in fig. 5.

	1	2	1 + P		2 + P	
			exp.	obs.	exp.	obs.
Dipyridamole	14	20	44	49	50	59
Theophylline	17	21	37	44	41	64

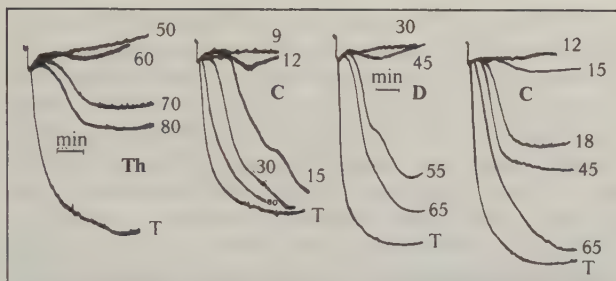
1: Dipyridamole 5 $\mu\text{g/ml}$ or theophylline 20 $\mu\text{g/ml}$.

2: Dipyridamole 25 $\mu\text{g/ml}$ or theophylline 100 $\mu\text{g/ml}$.

P: Prostacyclin 30×10^{-11} g/ml with dipyridamole and 20×10^{-11} g/ml with theophylline.

exp.: expected obs.: observed

The experiments shown in fig. 6 and table II utilize the lability of prostacyclin in aqueous solutions to demonstrate the prolonging effect of dipyridamole and theophylline on the effect of PGI_2 on platelet aggregation. The results in table II are achieved if one assumes a halflife of 3 minutes for PGI_2 (Cho and Allen 1978) and first order kinetics. In these experiments slight platelet aggregation could be induced in control platelets after 12 minutes while this was not possible in theophylline treated platelets before 60 minutes after PGI_2 addition. For dipyridamole the corresponding



The numbers indicate time for thrombin (0.08 U/ml) addition in minutes elapsed after addition of 2×10^{-9} g/ml PGI₂ to a suspension of washed acetylated platelets (C) and a suspension of washed acetylated platelets treated with 20 μ g/ml theophylline (Th) or 5 μ g/ml dipyridamole (D).

Figure 6. The prolongation of the effect of PGI₂ by theophylline (Th) and by dipyridamole (D).

Table II. Results of the experiments fig. 6 calculated from PGI₂ halflife of 3 minutes at the time when the inhibition of platelet aggregation corresponds to 6×10^{-10} g/ml PGI₂.

	Time (min)	Expected conc. PGI ₂ (g/ml)	Observed halflife (min)	Agent halflife Control halflife
Theophylline ...	60	2×10^{-15}	34.5	5
Control	12	1.25×10^{-10}	6.9	
Dipyridamole ..	45	6×10^{-14}	25.9	3
Control	15	6.3×10^{-11}	8.6	

figures were 15 and 45 minutes. The results clearly demonstrate a prolonging effect and not just a potentiating effect. The potentiating effect demonstrated in fig. 5 could never potentiate a concentration of 2×10^{-15} g/ml to the effect corresponding to 6×10^{-10} g/ml. That the drugs themselves did not have a stabilizing effect on PGI₂ was demonstrated by the fact that the supernatant from platelets suspended in the drugs did not have any inhibitory effect on platelet aggregation 20 minutes after PGI₂ addition.

The experiments in fig. 6 were also done with the concentration of the drugs increased 5 times. This increased the halflife of the effect further. E.g. a dipyridamole concentration of 25 μ g/ml increased the

$t_{1/2}$ by a factor 3.7 compared to control. However, this figure should be taken with some caution, since the halflife of the effect of PGI_2 in the control experiments varied a great deal although the platelets were from the same volunteer. This indicates that platelets may contain some phosphodiesterase inhibitory activity which is not completely washed out. This could be due to tea, coffee, the large dose of ASA or another inhibitor of phosphodiesterase present in the platelets of the volunteer.

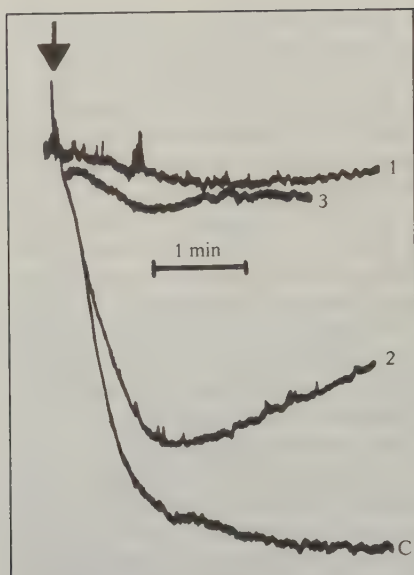
The demonstrated relationship between prostacyclin and phosphodiesterase inhibitors is independent of TXA_2 synthesis, since the used platelets were acetylated. The results are therefore still another indication that PGI_2 exerts its effect on platelets by stimulating the enzyme adenylate cyclase and they render support to the statement of Moncada and Korb (1978b) that phosphodiesterase inhibitors act as antithrombotic agents by potentiating endogenous prostacyclin. It also explains why these agents seem to be more effective in some »in vivo« situations than »in vitro« laboratory tests would seem to indicate (Packham and Mustard 1977, Buchanan and Hirsh 1978). It is possible that the effect of dipyridamole »in vivo« is that of prolonging the effect of PGI_2 from for example lungs, so that the effect is preserved through the circulation. The demonstrated effect may also have other clinical implications. Prostacyclin has been administered to man and may soon be used in the treatment of diseases (Gryglewski et al 1978d, Szczeklik et al 1978a, b, 1979a). Phosphodiesterase inhibitors may then be useful in prolonging the intervals between doses of prostacyclin.

STABILITY IN PLASMA

Right from the discovery of PGX it was noted that prostacyclin was unstable compared to classical prostaglandins. The inhibitory effect on platelet aggregation disappeared within 10 minutes at 37°C $\text{pH}=7.5$ and within 15 seconds upon boiling. It only disappeared slowly kept on ice and could be preserved for days in dry acetone at $\pm 20^\circ\text{C}$ (Moncada et al 1976a, 1977a, Gryglewski et al 1976). Cho and Allen (1978) studied the chemical stability of prostacyclin in different buffered aqueous solutions, demonstrating that it is

very unstable at acidic pH with an increasing halflife as pH becomes more alkaline. Dusting et al (1978) found a halflife of 3 minutes in the dog assaying PGI_2 by its muscle relaxing properties. They were of the opinion that the inactivation of PGI_2 in blood was by a chemical rather than an enzymatic mechanism, since they expected a chemical halflife of 2.8 minutes.

There is, however, some disagreement upon the stability of prostacyclin in plasma. Pedersen (1978) found that if blood samples were collected in test tubes containing PGI_2 , the anti-aggregating activity is retained for up to 2 hours at room temperature. He therefore suggested that PGI_2 was protected against degradation when kept in whole blood or plasma. The experiment shown in fig. 7 demonstrates that the effect of PGI_2 is preserved within the platelets in PRP (Jørgensen et al 1979a). Normal PRP containing 250×10^9 platelets/l was used. Tracing C shows the ADP ($6.7 \mu\text{M}$) induced aggregation of $150 \mu\text{l}$ PRP + $150 \mu\text{l}$ PPP. 4 ng/ml PGI_2 was then added to some PRP. After 70 minutes the ADP induced aggregation of $150 \mu\text{l}$ of this PRP + $150 \mu\text{l}$ PPP was still completely abolished (1). 64 minutes after PGI_2 addition some of the PRP was centrifugated at 1500 g for 5 minutes. $150 \mu\text{l}$ of the supernatant, which contained less than 1×10^9 platelet/l, was added to $150 \mu\text{l}$ of PRP which had not been exposed to PGI_2 . The ADP induced aggregation of this platelet suspension was only slightly inhibited (2). The platelets in the sediment were easily resuspended in PPP which had not been exposed to PGI_2 . In this suspension containing 116×10^9 platelets/l ADP induced aggregation was still greatly inhibited. Although plasma still seemed to have some antiaggregatory activity after 70 minutes, the experiments show that most of the inhibitory activity after 70 minutes is present within the platelets. Since this inhibitory activity disappears much faster in washed platelets, plasma may contain a factor or factors which preserve the effect of PGI_2 within platelets. This is the reason for using washed platelets in the experiments with phosphodiesterase inhibitors. This activity could be the explanation of the long duration of antiaggregatory activity seen when prostacyclin is administered to humans (Gryglewski et al 1978d, Szczeklik et al 1978a, b).



Arrow indicates addition of ADP in a final concentration of $6.7 \mu\text{M}$.

C: Control experiment, PRP + PPP, 1 + 1.

1: 70 min after PGI_2 addition.

2: Normal platelets suspended in the supernatant of plasma from experiment 1.

3: Platelets from experiment 1 suspended in normal plasma.

Figure 7. Plasma preserves the effect of PGI_2 in platelets.

The problem on the stability of prostacyclin in plasma is still unsolved. It is possible that PGI_2 is degraded to another antiaggregatory principle in plasma (Borda et al 1980, Gimeno et al 1980).

BIPHASIC EFFECT OF PROSTACYCLIN (V, Jørgensen et al)

While making standard curves for determination of PGI_2 concentrations by bio-assay it was discovered that prostacyclin has a biphasic effect on platelet aggregation (V, Jørgensen et al). In the range of 10^{-11} to 10^{-10} g/ml PGI_2 enhanced the aggregation of both washed platelets and PRP. The effect was easily demonstrated in platelets with blocked thromboxane synthesis, but more difficult to demonstrate in normal platelets. A similar biphasic effect of PGI_2 has been demonstrated

on the perfusion pressure of rat and rabbit heart (Karmazyn et al 1978). A possible explanation could be that prostacyclin may have some agonistic effect on the endoperoxide/thromboxane receptor apart from its effect on the adenylate cyclase. This could explain why the effect more easily can be demonstrated in vessel wall and acetylated platelets, where TXA_2 does not occur and the receptors therefore are free. PGE_2 also has a biphasic effect on platelet aggregation (Shio and Ramwell 1972).

Chapter IV

MODULATION OF PRODUCTION OF THROMBOXANE AND PROSTACYCLIN

The prostacyclin production stimulating factor

The »in vivo« regulation of endothelial prostacyclin production is very scarcely understood. Thrombin, trypsin, arachidonate, estradiol and mechanical trauma all stimulate the prostacyclin production of endothelial cells (Baenziger et al 1977, Weksler et al 1977, 1978, Moncada et al 1979, Silberbauer et al 1979a, b, Buchanan et al 1979, Hong 1980, Chang et al 1980).

MacIntyre et al (1978) found that cell-free plasma contained a factor which stimulated endothelial prostacyclin production. The factor was found in rat and pig plasma as well as human plasma prepared with citrate, EDTA or heparin. It was also found in serum and was still present after freezing of plasma. It has protein character since it was heat labile (56°C , 30 min) and non-dialysable. They also demonstrated that the factor was not albumin.

As described in chapter II the cause of the uremic bleeding tendency is not understood. Remuzzi et al (1977, 1978a, b, 1979a) found increased prostacyclin production in venous specimen from uremic patients, the capacity being greatest in patients with the longest bleeding time. They then demonstrated that uremic plasma had an increased stimulating effect on the prostacyclin production of »exhausted« rat aortic rings compared to normal plasma. Increased prostacyclin availability of vascular tissue has been demonstrated in experimentally induced uremia in rats (Silberbauer et al 1978b). It therefore seems that uremic plasma may

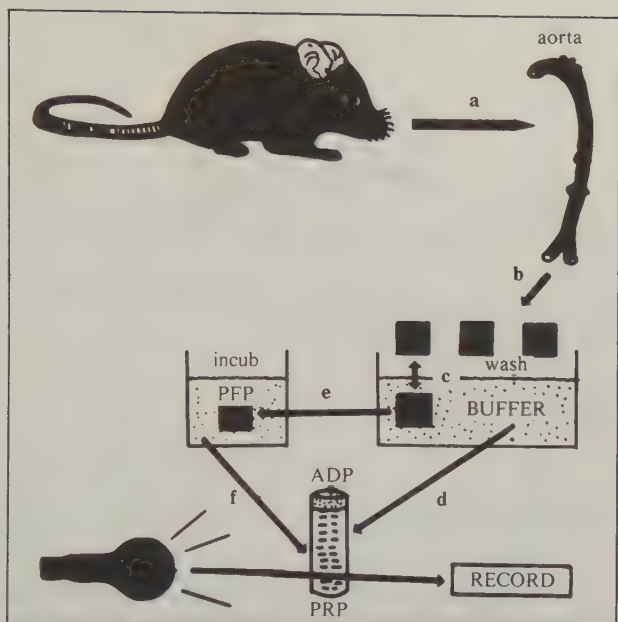
contain an increased amount of a prostacyclin production stimulating factor, which is involved in the uremic bleeding tendency.

It has also been mentioned in chapter II that platelets seem to play an important role in the hemolytic uremic syndrome (HUS). Remuzzi et al (1978c, 1979c) reported of two patients with HUS in which plasma exchange corrected the clinical disorder. Venous specimen from the patients did not release prostacyclin spontaneously before the plasma exchange while the release became normal after the plasma exchange. The patients plasma had a markedly reduced capacity compared to normal plasma in stimulating prostacyclin production of »exhausted« rat aorta and cultured pig aorta endothelial cells (Remuzzi et al 1980). In one of the patients the prostacyclin production stimulating capacity of plasma was still low one year after recovery. This patient had two daughters with normal prostacyclin production stimulating capacity, while this capacity was consistently low in two sons who had no history of microangiopathic disorders (Remuzzi et al 1979b).

THE PRESENT AUTHORS INVESTIGATIONS (VI, Jørgensen and Pedersen)

A 2 year old girl who had had thrombocytopenia, hemolytic anemia and acute renal failure for 5 days following a week with gastroenteritis was demonstrated to be deficient in prostacyclin production stimulating factor (PSF) determined as demonstrated in fig. 8 and 9. The patient was treated with blood and plasma exchange and recovered rapidly as demonstrated in fig. 10. After the blood and plasma exchange the patient's plasma was found to contain PSF as late as 12 days after the onset of acute renal failure, but on day 15 and after recovery the plasma was again deficient in PSF. The patient's mother also had PSF deficiency while the father's plasma contained PSF. There were no brothers or sisters.

PSF was demonstrated in a random plasma sample which had been frozen at -70°C for 2 years and also in serum from 1,000 blood donors. PSF was not antithrombin-III. There was no significant difference in prostacyclin production from arachidonic acid of a



- a: The rat is bled under ether anaesthesia and the aorta dissected free.
- b: The aorta is cut to pieces weighing 15 mg wet weight.
- c: The aorta pieces are washed in TRIS buffer.
- d: Every 1/2 hour the aorta is incubated for 10 min in TRIS buffer. 50 μ l is added to 250 μ l PRP and aggregation induced. c is continued until d shows no inhibition of aggregation.
- e: The piece of washed aorta is then incubated for 10 min, 37°C in 300 μ l platelet free plasma.
- f: 50 μ l of this incubate (e) is added to 250 μ l PRP and aggregation induced.

Figure 8. *Determination of the prostacyclin production stimulating factor.*

human umbilical vascular microsome suspension whether it was incubated in normal or PSF deficient plasma. PSF therefore presumably increases the substrate availability to the cyclooxygenase system in the endothelial cells. Its effect is therefore probably mediated through stimulation of the phospholipase. The experimental drug Bay g 6575 (Vermynen et al 1979) and oral contraceptives (Sinzinger et al 1980) have been shown to increase prostacyclin production in humans, and some observations indicate that dipyridamole also has a similar effect (Masotti et al 1979b, Ashida et al 1980).

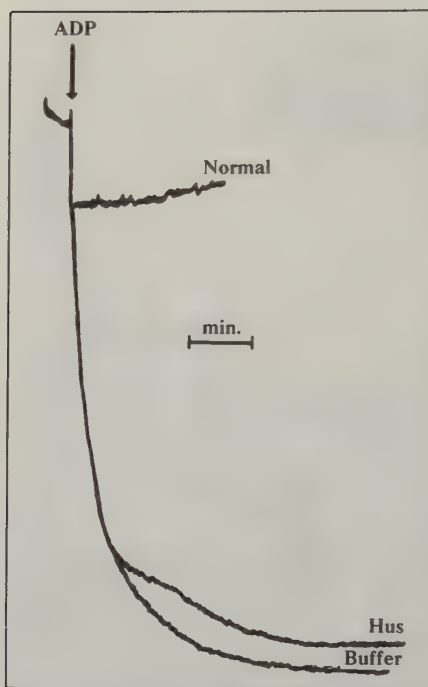
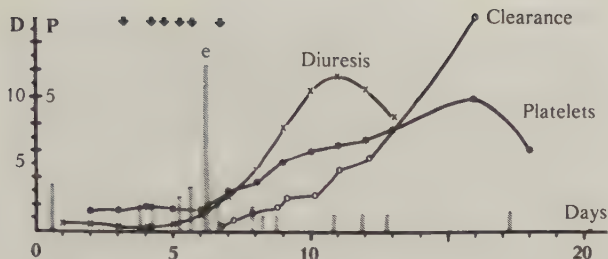


Figure 9. Resulting aggregation tracings from a normal healthy person and the patient with hemolytic uremic syndrome determined as described in fig. 8.

Phospholipase

Since platelets contain virtually no free arachidonic acid (Marcus et al 1969) and plasma only very small amounts (Hagenfeldt and Wahren 1975) it is assumed that release of arachidonic acid from phospholipids plays a key role in the regulation of platelet prostaglandin synthesis (Bills et al 1978). It is believed that this critical step is catalyzed by phospholipase C which hydrolyses phosphatidyl inositol (Rittenhouse-Simmons 1979) and by phospholipase A_2 which frees arachidonic acid from phosphatidyl choline (Derksen and Cohen 1975, Schoene and Iacono 1975, Bills et al 1976, Flower and Blackwell 1976, Rittenhouse-Simmons et al 1977, Blackwell et al 1977). The phospholipase A_2 has a specificity for 20 carbon fatty acids in 2-position of



Abcissa: Days after admission to hospital.

D: Number $\times 10^2$ indicates diuresis in ml/24 hours (x-line). Number indicates creatinine clearance in ml/min $\times 1.73 \text{ m}^2$ (open circle line).

P: Number $\times 10^8$ indicates platelets/ml (asterisk line). Number $\times 10^2$ indicates ml plasma given to the patient (hatched columns).

arrows: Hemodialysis treatment.

e: Plasma exchange.

Figure 10. *The course of a case of hemolytic uremic syndrome.*

phospholipids (Bills et al 1977). It has been demonstrated that low cAMP, high Ca^{++} and thrombin stimulate phospholipase activity (Derksen and Cohen 1975, Russell and Deykin 1976, Lapetina et al 1977, Gerrard et al 1977, Rittenhouse-Simmons and Deykin 1978, Bills et al 1978). Thus if TXA_2 increases free cytosol Ca^{++} and indirectly decreases cAMP it may have a positive feed back for its own production.

The mechanisms governing endothelial phospholipase activation are only very scarcely known. Piper and Vane (1971) proposed that disturbances of cell membranes are the stimuli which lead to synthesis of prostaglandins. Markelonis and Garbus (1975) reviewed the literature on their suggestion that prostaglandin synthesis is initiated in response to intracellular conditions which indicate an actual or potential deficit in the capability for maintaining cellular levels of ATP. They proposed that a fall in the intracellular oxygen would be followed by a decreased capacity for oxidative phosphorylation of mitochondria. The deteriorating mitochondrial function would allow release of sequestered Ca^{++} which would activate mitochondrial and microsomal phospholipases, which again would liberate arachidonic acid from membrane phospholipids.

Glucocorticosteroids (GCS) are believed to inhibit

prostaglandin production by inhibiting the phospholipase enzymes, since the inhibitory effect can be overcome by addition of arachidonic acid (Gryglewski et al 1975, Hong and Levine 1976, Lewis et al 1979). Current evidence, however, suggests that the inhibition of prostaglandin biosynthesis by GCS is dependent upon m-RNA and protein synthesis (Danon and Assouline 1978, DiRosa and Persico 1979). Since platelets are incomplete cells without a nucleus, such a mechanism of action would seem impossible in platelets while the endothelial phospholipase could be inhibited in this manner.

There is some controversy about the effects of GCS on platelet aggregation. Most investigators find that very high concentrations inhibit platelet aggregation »in vitro« (Pierce et al 1974, Nelson and Taylor 1975, Cazenave et al 1976, Sanders et al 1976). However, even very high doses do not seem to inhibit »in vivo« platelet aggregation in cats (Vaage 1978). The following investigations were therefore undertaken to find out if GCS inhibit platelet aggregation by inhibiting platelet thromboxane formation and to test the assumption that GCS inhibit endothelial prostacyclin production.

THE PRESENT AUTHORS' INVESTIGATION (VII, Jørgensen and Stoffersen)

Hydrocortisone inhibition of platelet prostaglandin metabolism

Hydrocortisone (Solu-Cortef®, Upjohn s.a., Puurs, Belgium) was added to PRP containing normal and acetylated platelets in various concentration and incubated at 37°C for 30 minutes. Aggregation was then induced with ADP. As seen in fig. 11 0.8 mg/ml completely blocked the secondary aggregation induced by 1.6 μ M ADP in normal platelets, while the inhibition of ADP (4.5 μ M) induced aggregation of acetylated platelets was very slight requiring very large concentrations. As shown in fig. 12 hydrocortisone (3 mg/ml) also inhibited the thrombin induced aggregation of washed platelets. This inhibition could, however, be overcome by small amounts of arachidonic acid. Table III shows the MDA formation of normal platelets upon incubation for 10 minutes with thrombin or arachidonic acid.

It is clearly seen that hydrocortisone inhibits thrombin induced MDA formation but not arachidonate induced MDA formation.

These results clearly demonstrate that the inhibitory effect of hydrocortisone on platelet aggregation is

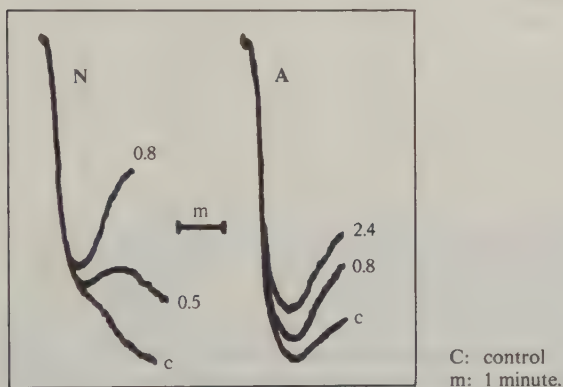


Figure 11. The influence of hydrocortisone of the ADP induced aggregation in PRP of normal (N) and acetylated (A) platelets.

Numbers indicate concentration of hydrocortisone in mg/ml.

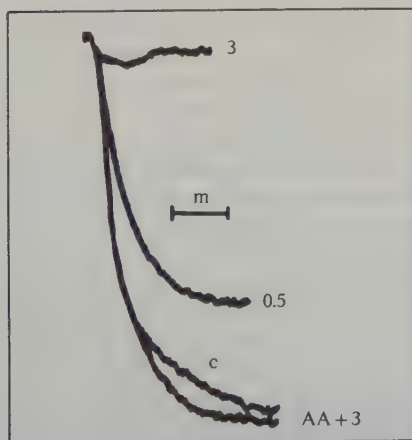


Figure 12. The inhibition of thrombin induced aggregation of normal washed platelets by hydrocortisone and its reversal by arachidonic acid.

Numbers, c and m as fig. 11. AA + 3: 0.15 mM arachidonate + 3 mg/ml hydrocortisone.

Table III. *Malondialdehyde (MDA) generation upon thrombin and arachidonic acid stimulation of platelet rich plasma containing various concentrations of hydrocortisone.*

Incubation with	Hydrocortisone mg/ml	MDA production nmol/3 × 10 ⁸ platelets
Thrombin (1.5 U/ml)	0.0	1.11
	1.0	0.95
	3.0	0.00
Arachidonic acid (1 mmol/l)	0.0	1.95
	1.0	2.35
	3.0	2.10

mediated through inhibition of platelet thromboxane production, since MDA formation in platelets is catalyzed by the thromboxane synthetase enzyme (McMillan et al 1978). The results also demonstrate that the inhibition is mediated through inhibition of arachidonic acid release to the prostaglandin synthetase systems.

Hydrocortisone inhibition of endothelial prostacyclin production

Pieces of rat thoracic aortic tissue (RAT — 15 mg wet weight), were washed in various concentrations of hydrocortisone in TRIS buffer (pH = 7.4) for 30 minutes. The RAT was then incubated in 300 µl TRIS buffer containing the same concentration of hydrocortisone. 50 µl of the incubate was then added to 250 µl acetylated PRP and ADP (4.5 µM) aggregation induced. As seen in fig 13, 0.8 mg/ml hydrocortisone completely inhibited the spontaneous prostacyclin release from the RAT. In three consecutive experiments the spontaneous prostacyclin release was 60-96 pg/mg rat aortic tissue. Hydrocortisone at a concentration of 0.4 mg/ml decreased this release to 18-30 pg/mg. 0.8 mg/ml and 3 mg/ml completely inhibited the spontaneous prostacyclin release of RAT. However, when the piece of RAT which had been incubated with 3 mg/ml hydrocortisone was incubated with 1 mM arachidonic acid and 3 mg/ml hydrocortisone, the spontaneous prostacyclin release was restored (fig. 14).

These experiments clearly demonstrate that hydrocortisone inhibits endothelial prostacyclin release through inhibition of arachidonic acid release from the

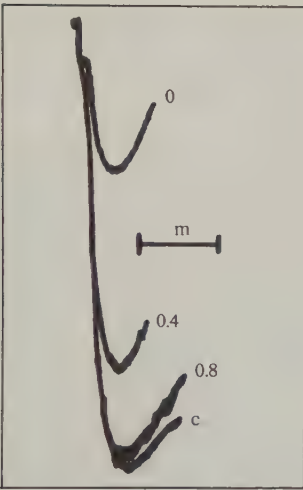
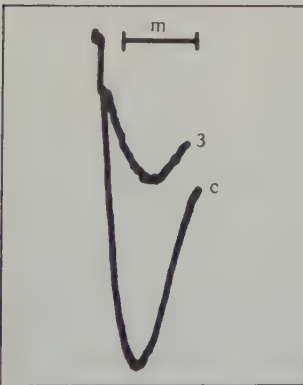


Figure 13. *Hydrocortisone inhibition of vascular prostacyclin production.*

The aggregation of 250 µl acetylated PRP added 50 µl of an incubate containing a piece of aortic tissue and hydrocortisone. The numbers indicate the concentration of hydrocortisone in mg/ml. 3 mg/ml gave the same tracing as 0.8 mg/ml.

c: the 250 µl PRP added 50 µl TRIS-buffer.



3: The aggregation of 250 µl PRP added 50 µl of an incubate containing 3 mg/ml hydrocortisone, 1 mM arachidonic acid and a piece of aortic tissue.

c: The aggregation of 250 µl PRP + 50 µl 1mM arachidonic acid in TRIS-buffer.

Figure 14. *The reversal of hydrocortisone inhibition of vascular prostacyclin production by arachidonic acid.*

endothelial phospholipids. The results are in accordance with the results of Blajchman et al (1979). They demonstrated that hydrocortisone shortened bleeding time in rabbits. The effect could be reversed by arachidonic acid and by prostacyclin.

In the experiments presented here hydrocortisone seemed more effective in inhibiting spontaneous prostacyclin release, than in inhibiting platelet MDA release. Although the results are in accordance with the concept that the endothelial cells are more sensitive to hydrocortisone than platelets, they do not warrant such a conclusion, since the spontaneous prostacyclin release of pieces of rat aortic tissue cannot be compared with thrombin induced MDA production of human platelets. The concentrations used in these experiments are also higher than usually seen in clinical practice. Thong et al (1978) decreased mean bleeding time from 6.6 min. to 5.9 min. in 12 healthy volunteers by giving 80 mg of prednisone orally for 2 days. This could indicate a decrease in prostacyclin production in humans but the reduction was not significant.

The effect of albumin

Hypoalbuminemia is a key feature of the nephrotic syndrome in which an increased tendency towards thrombosis has been postulated (Berlyne and Mallick 1969, Kanfer et al 1970, Kendall et al 1971, Clarkson et al 1976). Although this is due to many other factors (Thomsen et al 1974, Kaufmann et al 1978, Thaler et al 1978, Jørgensen and Stoffersen 1979b, c) it could also be due to lack of albumin inhibition of platelet aggregation. Albumin has been shown to inhibit platelet aggregation, an effect thought to be due to the great affinity of arachidonic acid to albumin (Goodman 1958, Silver et al 1973). Indeed hyperaggregable platelets have been found in children with the nephrotic syndrome (Bang et al 1973).

The following investigations were therefore undertaken to find out if albumin inhibited platelet aggregation by inhibiting platelet prostaglandin metabolism and also to test the assumption that the hyperaggregability of platelets seen in the nephrotic syndrome is due to the low level of serum albumin in these patients.

THE PRESENT AUTHORS' INVESTIGATIONS (VIII, Jørgensen and Stoffersen)

Suspensions of acetylated and normal washed platelets were added to an equal volume of albumin (Albumin purified, Behringwerke, Germany) in TRIS buffer pH = 7.4 to yield the desired final concentration. Aggregation was induced with thrombin and pH measured before and after aggregation. The aggregation of acetylated and normal platelets were recorded simultaneously.

As seen in fig. 15 albumin had an inhibitory effect on both acetylated and normal platelets, but the inhibitory effect was much more pronounced in normal than in acetylated platelets. All pH measurements were between 7.35 and 7.40. Fig. 16 demonstrates that part of the inhibition of platelet aggregation produced by albumin in normal platelets can be overcome by addition of 1 mM arachidonic acid, while arachidonic acid increased the inhibition in acetylated platelets. These observations support the hypothesis that albumin inhibits platelet aggregation by depriving the cyclooxygenase of its substrate arachidonic acid. However, since albumin also inhibited the aggregation of acetylated platelets, albumin also has another mechanism of inhibition of platelet aggregation.

Five patients with various degrees of hypoalbuminemia because of glomerular renal disease who had not taken any drugs for a period of three weeks were tested for ADP induced aggregation of PRP. Two samples were collected at the same time from each patient. A small amount of a 20 % albumin solution in saline was added to one sample to bring the albumin concentration to between 40 and 50 g/l. The same amount of saline was added to the other sample. The two samples were prepared and studied simultaneously. Aggregation was induced by adding 0.4, 0.5, 0.64, 0.8, 1.0, 1.25, 1.6, 2.0, 2.5, 3.2, 4.0, and 5 μ M ADP and finding the minimal final ADP concentration necessary to induce a welldefined secondary wave of platelet aggregation (ADP_{min}). The results are given in table IV.

The five patients with a nephrotic syndrome had a lower ADP_{min} than five age and sex matched controls ($p < 0.05$). If the albumin level was raised the ADP_{min} rose significantly to values not different from the controls.

Other reports have now confirmed the hyperaggre-

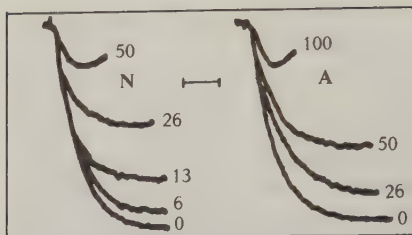
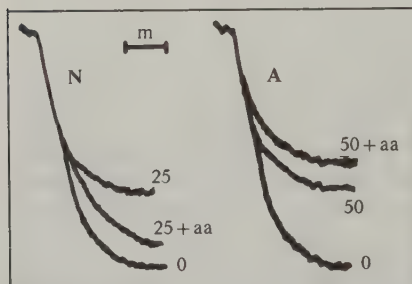


Figure 15. The effect of various albumin concentrations on thrombin induced aggregation of non-aspirin treated (N) and aspirin treated (A) washed platelets.

The numbers indicate the concentration of albumin in g/l.



N, A, and numbers as fig. 15.

aa: Added 1 mM arachidonic acid.

Figure 16. The reversal of albumin inhibition of platelet aggregation by arachidonic acid in normal platelets.

gability of platelets in the nephrotic syndrome with low levels of albumin. It has also been confirmed that the hyperaggregability can be corrected by addition of albumin as well »in vivo« as »in vitro« and that albumin inhibits platelet MDA formation (Yoshida and Aoki 1978, Remuzzi et al 1979d).

Cyclooxygenase activity

It is now generally accepted that acetylsalicylic acid (ASA) and most other non-steroid anti-inflammatory drugs exert their effects through inhibition of prostaglandin biosynthesis (Vane 1971, Moncada et al 1973, Vane 1976, 1978). It has long been known that ASA inhibits platelet function, that it is effective in very small doses, and that the effect lasts for days (Gast 1964, Weiss et al 1968, Evans et al 1968, O'Brien et al 1970).

Smith and Willis (1971) and Hamberg et al (1974a) demonstrated that ASA blocks the conversion of

Table IV. Serum albumin and ADP_{min} (see text) of 5 patients with a nephrotic syndrome before (A) and after (B) albumin is added to the sample. Also shown is the ADP_{min} and serum albumin of 5 sex and age matched controls.

		Patient no.				
		1	2	3	4	5
Albumin g/l	A:	18	20	15	8	10
	B:	41	34	51	45	35
ADP _{min} μM	A:	1.25	1.0	0.64	0.50	1.6
	B:	5.0	1.6	5.0	5.0	2.5
Control ADP _{min}	A:	2.5	4.0	2.5	4.0	4.0
Control albumin	A:	41	47	43	45	46

arachidonic acid to endoperoxides. It has been demonstrated utilizing ³H-ASA that ASA blocks the cyclooxygenation properties of the prostaglandin endoperoxide synthetase by irreversibly acetylating the protein enzyme (Roth et al 1975, Roth and Majerus 1975, Rome 1976).

³H-ASA has also been used to determine the sensitivity of platelets to oral ASA ingestion. A dose of 650 mg inactivated more than 95 % of the enzyme and as little as 20 mg inactivated 61 %. A 325 mg dose inhibited 89 % of the enzyme for 2 days. The sensitivity of platelet cyclooxygenase to ASA was found to be 31-fold greater than the ASA sensitivity of sheep seminal vesicles (Burch et al 1978a). A single dose of 600 mg inhibits arachidonic acid induced platelet aggregation, TXB₂ generation and malondialdehyde (MDA) formation for 2-4 days and pre-ASA values are not reached before 8-13 days. Since addition of 10-25 % normal platelets to ASA treated platelets restores arachidonate induced aggregation, ASA also has an effect on the megakaryocytes (Bye et al 1979, Szczeklik et al 1979b, Cerkus et al 1980).

Baenziger et al (1977, 1979) found that cultured human arterial smooth muscle cells, venous endothelial cells and skin fibroblasts were 14-44 times less sensitive to ASA inactivation compared to platelets. Using ³H-ASA Burch et al (1978b) found that microsomes from human aortas and coronary arteries were approximately 1/250 as sensitive to ASA as intact platelets and 1/60 as sensitive as platelet microsomes.

Doses below 15 mg/kg suppress arachidonate induced platelet aggregation in rabbits without inhibiting prostacyclin production while doses above 25 mg/kg also inhibited vascular prostacyclin production (Basista et al 1978). Arachidonate infusion into cats and rabbits produced slight disaggregation of platelets. This disaggregating effect is potentiated by a small dose of aspirin but blocked by a larger dose (Korbut and Moncada 1978).

Although 5 μ M ASA only inhibited about 10 % of the PGI₂ production of cultured arterial cells in the report of Baenziger et al (1977), Jaffe and Weksler (1979) found that the same concentration of ASA inhibited the arachidonic acid stimulated prostacyclin production of cultured endothelial cells. However, when endothelial cells which had been incubated with a high concentration of ASA were washed free of the ASA, the prostacyclin production returned to control values within 35 hours. This recovery was depended on de novo protein synthesis since cycloheximide inhibited recovery. One great difference in platelet and endothelial cyclooxygenase is that platelets are incomplete cells which do not regenerate new enzyme once it has been destroyed, while endothelial cells do.

Measurements of bleeding time have confirmed the difference in ASA sensitivity of platelets and endothelial cells (Chapter VI). Masotti et al (1979) reported that 3.5 mg/kg ASA was the dose most likely to produce a consistent inhibition of platelet aggregation and only slight inhibition of platelet aggregation.

Experimental evidence shows that a large dose of ASA can be thrombogenic. While 0.1 mM ASA blocked platelet MDA formation, 1-2 mM enhanced thrombin-induced adherence of washed platelets to endothelial monolayer cultures (Czervionke et al 1978). The experimental thrombogenic effect of a high dose of ASA is lost if an interval of 2½ hours or longer elapses between vessel damage and drug administration (Kelton et al 1978).

From these observation it seems clear that ASA in doses usually used for antithrombotic purposes inhibits both TXA₂ and PGI₂ synthesis. This may be the reason for the inconclusive results in some studies using ASA as an antithrombotic drug (Elwood et al 1974, Elwood and Sweetman 1979, AMIS 1980).

In consistence with the results indicating better effect of low dose ASA Harter et al (1979) found a significant decrease in shunt thrombosis in chronic uremic hemodialysis patients given 160 mg ASA daily compared to placebo in a double-blind randomized study. Already in 1952 Craven was surprised that as little as 5 grains (325 mg) of ASA 5 times a week seemed effective as prophylaxis against secondary myocardial infarction.

Thromboxane synthetase

An effective specific thromboxane synthetase inhibitor would seem to be a better drug for antithrombotic purposes than the cyclooxygenase inhibitors. Great effort is therefore being carried out to find clinically usable specific thromboxane synthetase inhibitors. Benzylamin was the first compound found to inhibit the thromboxane synthetase (Moncada et al 1976b). Imidazole was soon after shown to be a thromboxane synthetase inhibitor and it is the basis for much research in this field (Moncada et al 1977c, Needleman et al 1977a, b). Nictindole and quite a few endoperoxide and thromboxane analogs have thromboxane synthetase inhibitor properties although some also seem to be receptor antagonists for TXA₂. (Gorman et al 1977a, Gryglewski et al 1977, Sun 1977, Diczfabeasy and Hammarström 1977, Fitzpatrick et al 1978, 1979, Grodzińska et al 1979, Nicolaou et al 1979, le Breton et al 1979).

Prostacyclin synthetase

The monoamine oxidase inhibitor tranylcypromine is a prostacyclin synthetase inhibitor and has been used as such in many experimental situations (Gryglewski et al 1976, Weksler et al 1977, Kelton et al 1978, Bourgain et al 1979). Platelet β -thromboglobulin also seems to be an inhibitor of prostacyclin synthetase (Hope et al 1979).

It is, however, the lipoxygenated arachidonic acids which have aroused most interest. 15-hydroperoxyarachidonic acid (15-HPAA) is a potent inhibitor of the prostacyclin synthetase with an IC₅₀ of 0.43 μ g/ml (Moncada et al 1976c, Gryglewski et al 1976, Moncada et al 1977, Bourgain 1980) an activity shared by several hydroperoxides of unsaturated fatty acids (Salmon et al 1978, Ham et al 1979, Weiss et al 1979). This interest is

due to the increased content of lipid peroxides found in atherosclerotic vessel wall and the theories connecting atherogenesis and the process of aging with lipid peroxidation (Glavind et al 1952, Harman 1973, Wilson 1976, Filipovis and Rutemöller 1976). Increased lipid peroxidation is also found in E-vitamin deficiency (Dam 1957) and the prostacyclin generation of rat aortas is significantly reduced if the rats are fed a vitamin E deficient diet (Okuma et al 1980). These observations may be the rational basis for the use of anti-oxidants in prevention and treatment of atherosclerosis and thrombosis.

Prostacyclin generation is reduced in arteries from rabbits with experimental atherosclerosis and in human atherosclerotic plaques (Dembińska-Kieć et al 1977, 1979a, D'Angelo et al 1978). In early experimental atherosclerosis in rabbits it has been found that the platelets have an increased sensitivity to the antiaggregatory effect of prostacyclin (Zmuda et al 1977, Gryglewski et al 1978a, Dembińska-Kieć et al 1979b). This probably reflects a decreased in vivo prostacyclin concentration resulting in an increased sensitivity of the adenylate cyclase to prostacyclin. PGE₁ stimulation of the adenylate cyclase results in a decreased sensitivity of the adenylate cyclase to prostacyclin (Gorman and Hopkins 1980). An increased TXA₂ formation and ADP sensitivity was also demonstrated in the experimental atherosclerotic animals (Zmuda et al 1977, Gryglewski et al 1978a, Dembińska-Kieć et al 1979b). This is probably also due to decreased PGI₂ concentration resulting in low levels of cAMP in the platelets, since high cAMP levels inhibit TXB₂ formation. Cultured smooth muscle cells from atherosclerotic rabbit aorta also generate decreased amounts of prostacyclin (Larue et al 1980). It has also been suggested that the great resistance to atherosclerosis of rats is due to the high prostacyclin producing capacity of their arteries (Villa et al 1977a). The resistance of dipyridamole treated baboons to atherosclerosis also indicates a role of PGI₂ deficiency in atherogenesis (Harker et al 1976).

Eicosapentaenoic acid

The suggestion that eicosapentaenoic acid (EPA) might be antithrombotic came as a result of long-standing studies by Dyerberg and Bang (1979a) in Eskimos living

on the north-west coast of Greenland. These hunters and fishermen have a very low incidence of acute myocardial infarction and they have a bleeding tendency (Høygaard 1940, Annual Greenland reports 1963-1976, Harvald 1974, Dyerberg and Bang 1980). It was found that the ratio polyunsaturated/saturated fatty acids in the plasma of Eskimos did not differ significantly from this ratio in Caucasian Danes. However, a shift from n-6 to n-3 fatty acids was found in the Eskimos (Dyerberg et al 1975). This distribution of the polyunsaturated fatty acids in the eskimo plasma lipids was due to their diet (Bang et al 1976).

After the discovery of prostacyclin it was demonstrated that EPA incubated with vessel wall resulted in the production of an antiaggregatory principle which was postulated to be a » Δ -17«-prostacyclin, while platelets do not convert EPA to a proaggregatory prostaglandin (Dyerberg and Bang 1978, Dyerberg et al 1978). Investigations on the platelet fatty acid content and platelet aggregation demonstrated that the decrease in aggregability of the eskimo platelets was due to the platelet content of EPA instead of arachidonic acid (AA). The bleeding time was longer in Eskimos than in Caucasian Danes and in contrast to Danes it was shortened by acetylsalicylic acid (Dyerberg and Bang 1979b).

» Δ -17«-prostacyclin has been synthesized from PGH_3 by aortic microsomes and has been shown to have properties very similar to PGI_2 (Neddelman et al 1979b). PGH_3 has been shown to be a good substrate for both the thromboxane and the prostacyclin synthetase yielding the end products TXB_2 and » Δ -17«-6-keto- $\text{PGF}_{1\alpha}$ (Smith et al 1979). It has still not been demonstrated chemically that vessel wall can convert EPA to this » Δ -17«-prostacyclin (PGI_3).

EPA inhibits platelet aggregation in many ways. It inhibits the aggregation induced by arachidonic acid, collagen, the endoperoxide analogue 46619, thrombin and the calcium ionophore A 23187 (Silver et al 1973, Whittaker et al 1979, Jakubowski and Ardlie 1979). PGH_3 is converted to PGE_3 and PGD_3 in plasma. While PGE_2 prevents the inhibitory effect of PGD_2 on platelet aggregation, PGE_3 does not inhibit the inhibitory effect of PGD_3 (Gryglweski et al 1979). This is the mechanism by which EPA increases cAMP in platelets.

It has been shown that EPA is not oxidized by cyclooxygenase from sheep vesicular glands to the same degree as AA at low peroxide tone (Culp et al 1979) and EPA is a poor substrate for this cyclooxygenase (Smith et al 1979). It also seems to be a poor substrate for the platelet cyclooxygenase (Hamberg 1980). Attempts to produce ^{14}C - PGI_3 by incubating ^{14}C -EPA with rabbit aortic rings and attempts to demonstrate conversion of EPA to PGI_3 by perfused hearts and kidneys have failed (Whittaker et al 1979). It has therefore been postulated that the antithrombotic properties of EPA are caused by inhibition of platelet aggregation alone. It has also been suggested that EPA may inhibit PGI_2 production of vessel wall.

Diet rich in n-3 fatty acids has been shown to have a protective effect on experimental acute cerebral ischaemia in cats and in experimental thrombosis in rats (Black et al 1979, Nordøy 1979, Hornstra and Hemker 1979). A change from n-6 to n-3 fatty acids with resultant decreased platelet aggregability and increase in bleeding time has been achieved in German and British volunteers by supplementing the diet with 20 ml cod liver oil daily for 6 weeks or by eating 500-800 g of mackerel for 6 days (Sanders et al 1980, Siess et al 1980). These experiments together with the observations in Eskimos clearly demonstrate the antithrombotic properties of the n-3 fatty acids, whatever the biochemical mechanism.

Chapter V

THE EXCHANGEABILITY OF PROSTAGLANDIN ENDOPEROXIDES BETWEEN PLATELETS AND ENDOTHELIAL CELLS

Both platelets and vascular tissue can utilize exogenously added endoperoxides for production of TXA_2 and PGI_2 respectively (Hamberg et al 1974a, 1975, Moncada et al 1976a, Gryglewski et al 1976). Vascular tissue converts exogenous arachidonic acid to prostacyclin with a low yield (0.5-1 %) while the conversion of endoperoxides is much higher (80-90 %) (Bunting et al 1976b, Salmon et al 1978). Platelets also convert endoperoxides almost exclusively (Hamberg and Samuelsson 1974). It therefore seems that the thromboxane

and prostacyclin synthetase enzyme systems have a great affinity for endoperoxides. If excess endoperoxides are produced by platelets during aggregation it therefore seems possible that the endoperoxides may be used by endothelial cells to produce prostacyclin.

Bunting et al (1976b) demonstrated that vascular rings from indomethacin treated rabbits generated prostacyclin when incubated with platelets and they postulated such a biochemical interaction between platelets and vessel walls as an important physiological feature. Similar experiments using indomethacin treated placental tissue also seem to demonstrate that this tissue produces prostacyclin from platelet derived endoperoxides (Myatt and Elder 1977). Cultured cells can produce prostacyclin from exogenously added endoperoxides (Marcus et al 1978), and the prostacyclin production of these cells increases when the cells are incubated with platelets stimulated with collagen (Nordøy et al 1978, Willems and van Aken 1979). It has also been demonstrated that the production of 6-keto $\text{PGF}_{1\alpha}$ by lysed aortic smooth muscle cells is stimulated by human platelet extracts and that further stimulation is observed when the thromboxane synthetase inhibitor imidazole is added to the system (Tansik et al 1978). All these observations seem to demonstrate that the low conversion rate of the endothelial cyclooxygenase can be bypassed by a higher conversion rate of the platelet cyclooxygenase.

This theory has, however, caused some controversial debate (Needleman 1979) and some observations indicate that such a biochemical platelet-endothelial interaction does not occur "in vivo". Vascular rings from thrombocytopenic and control rats release the same amount of prostacyclin (Villa et al 1977b), however, unstable endoperoxides could have been degraded during isolation of the vascular rings. Baenziger et al (1979) were unable to demonstrate utilization of platelet derived endoperoxides by cultured endothelial cells unless imidazole was present. Needleman et al (1979a) were unable to demonstrate release of endoperoxides from intact platelets to aortic microsomes unless the platelets were incubated with imidazole.

Using small pieces of rat aortic tissue (0.1 mg) Hornstra et al (1979) were unable to demonstrate any difference in prostacyclin production whether the

pieces were incubated in PRP or PPP and indomethacin treated tissue did not produce prostacyclin irrespective of whether it had been incubated in PPP or PRP even if the platelets in PRP were stimulated with collagen. They also found that neither normal nor EFA-deficient aortic tissue (obtained from rats deficient in essential fatty acids) produce more prostacyclin on incubation with collagen activated normal PRP than with collagen activated EFA-deficient PRP. They demonstrated that in earlier experiments with indomethacin treated vascular tissue, the indomethacin might have been removed from the endothelial cyclooxygenase upon incubation in PRP. They postulated that blood platelets do not provide endoperoxides for vascular prostacyclin production.

The following investigations were carried out to elucidate further if endothelial cells can utilize platelet derived endoperoxides for prostacyclin production and also to test the assumption that platelets can utilize endothelial derived endoperoxides.

THE PRESENT AUTHORS INVESTIGATIONS

Conversion of platelet produced endoperoxides by vascular tissue

A piece of rat aortic tissue (RAT — see chapter I) was washed in TRIS-buffer (pH = 7.4) and incubated in PPP for 45 minutes. 50 μ l of this PPP was added to 250 μ l PRP and aggregation induced with ADP (4 μ M). This aggregation was completely inhibited (fig. 17-N) indicating prostacyclin production by the RAT. Another RAT was washed in a saturated solution of ASA in buffer. This ASA-RAT was incubated with collagen (10 μ g/ml) in PPP for 45 min at 37° C. When 50 μ l of this PPP was added to 250 μ l PRP there was no inhibition of ADP induced aggregation (fig. 17-P), showing that this ASA-RAT did not produce prostacyclin. The same ASA-RAT was then incubated with collagen in PRP. After 40 minutes this incubate was centrifugated at 2000 g for 5 minutes. When 50 μ l of the supernatant was added to 250 μ l PRP, the ADP induced aggregation was somewhat inhibited (fig. 17-R). This experiment clearly demonstrates that pieces of whole rat aortic tissue can utilize prostaglandin endoperoxides produced in plate-

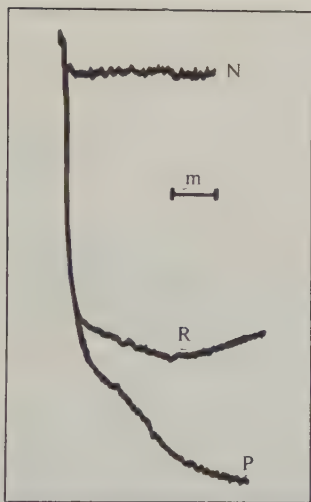


Figure 17. The ADP induced aggregation of 250 μ l PRP to which is added 50 μ l of an incubate of

N: RAT in PPP

P: ASA-RAT in PPP

R: ASA-RAT in PRP

Abbreviations: See text.

lets when their own cyclooxygenase has been blocked with ASA. The experiment does, however, indicate that the amount of prostacyclin that can be produced in this manner is very small compared to the amount produced from endothelial derived endoperoxides.

Hornstra et al (1979) concluded from their investigations that endothelial cells did not use platelet derived endoperoxides. The greatest difference in their experiments and the experiments presented here is that they used much smaller pieces of aortic tissue which may not have been able to produce measurable amounts of prostacyclin. Another difference is the use of rat platelets while human platelets were used in the present investigation.

Conversion of vascular produced endoperoxides by platelets

The low capacity of the vessel wall cyclooxygenase is the theoretical background for expecting endothelial utilization of platelet derived endoperoxides. If, how-

ever, platelet cyclooxygenase is blocked selectively, either by administration of a small dose of ASA (Masotti et al 1979, Jørgensen et al 1979b) or some time after administration of a large dose (Amezcuca et al 1979), the opposite could be expected, namely that platelets can utilize endothelial derived endoperoxides for thromboxane formation. This possibility was investigated in another set of experiments.

600 μ l PPP, PRP or ASA-PRP was incubated with 1 mM arachidonic acid (AA) after addition of various combinations of TRIS buffer, 30 μ l calf aortic microsomes (CAM) and 5 mM imidazole to a total volume of 750 μ l and the MDA generation measured. The results are given in table V.

The great reduction in MDA generation upon addition of the thromboxane synthetase inhibitor imidazole to platelets confirms earlier observations showing that MDA generation is an indication of thromboxane synthetase activity (McMillan et al 1978). The very low MDA generation by CAM in PPP shows that CAM did not give rise to significant MDA generation. The low MDA generation of ASA-PRP shows that the cyclooxygenase of the platelets was effectively blocked. The very high MDA generation of ASA-platelets incubated with CAM and the decrease in MDA generation upon imidazole addition clearly demonstrates that platelets readily utilize CAM produced endoperoxides for thromboxane formation. Small amounts of MDA were found in incubates where it was not expected. This is probably due to measurement inaccuracy and a small variability in the exact platelet number in both PPP and PRP.

In another series of experiments RAT was washed in a suspension of the prostacyclin synthetase inhibitor tranylcypromin (1 mg/ml) (TCM) and then incubated for 25 minutes in PPP with AA and TCM. 50 μ l of this PPP was then transferred to ASA-PRP and aggregation induced with ADP (fig. 18-T). Another RAT was washed in both ASA and TCM and then incubated in the same manner. When 50 μ l of this suspension was added to ASA-PRP and aggregation induced with ADP the aggregation occurred to a lesser extent (fig. 18-A). If the RAT was washed in buffer only and incubated without TCM the resulting aggregation was completely inhibited as shown in fig. 18-N.

Table V. MDA generation of different suspensions upon incubation with arachidonic acid (1 mM). Abbreviations see text.

PPP	0.62 nmol/ml
PPP + CAM	0.62 nmol/ml
PPP + CAM + imidazole	0.66 nmol/ml
PRP	1.41 nmol/ 3×10^8 platelets
PRP + imidazole	0.50 nmol/ 3×10^8 platelets
ASA-PRP	0.27 nmol/ 3×10^8 platelets
ASA-PRP + CAM	1.89 nmol/ 3×10^8 platelets
ASA-PRP + CAM + imidazole	0.84 nmol/ 3×10^8 platelets

This experiment seems to indicate that endoperoxides produced by the endothelial cyclooxygenase enhances the aggregation of ASA-platelets. Since minute amounts of prostacyclin can enhance platelet aggregation (V, Jørgensen et al) production of discrete amounts of prostacyclin could explain the results. It was therefore necessary to measure TXB₂ production in these experiments.

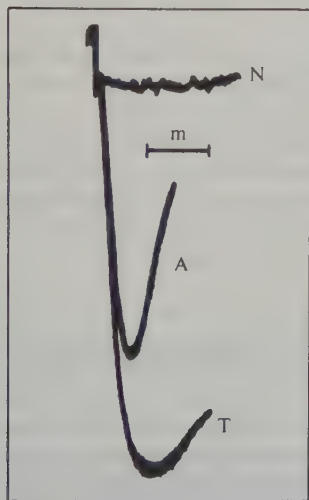


Figure 18. The ADP induced aggregation of 250 μ l ASA-PRP added 50 μ l of an incubate of
N: RAT with AA in PPP
T: TCM-RAT with AA in PPP
A: ASA-TCM-RAT with AA in PPP
Abbreviations: See text.

Five pieces of RAT were washed in TCM and incubated for 45 minutes with AA and TCM in PRP. After this TXB_2 was measured. Another 3 pieces of RAT were washed in ASA and then treated as the other five. In the incubates where RAT was pretreated with ASA, the TXB_2 concentrations were in the range of 0-1.2 pM/ml, while it was 4-7 pM/ml in the non-ASA treated incubates. This clearly demonstrates that the ASA treated platelets did utilize endoperoxides produced by RAT for thromboxane formation when the prostacyclin synthetase in the RAT was inhibited. Usually a stimulation of platelets resulting in secondary aggregations results in production of TXB_2 above 50 pM/ml. The degree to which platelets utilize endothelial derived endoperoxides in this experiment is therefore small.

In the series of experiments presented here it has been documented that platelets and endothelial cells mutually can utilize endoperoxides produced by the other part, but the experiments also seem to demonstrate that in intact cells this only occurs to a minor degree. It is, however, possible that there are situations, 'in vivo' where the capacity of the enzyme systems involved is restricted so that a platelet-endothelial interaction can occur through exchange of endoperoxides. Too little is, however, known about the 'in vivo' regulation of the enzyme systems. It has for example been demonstrated that endothelial damage facilitates endoperoxide conversion to prostacyclin (Hornstra et al 1978, Puré and Needleman 1979).

Chapter VI

ACETYSALICYLIC ACID AND BLEEDING TIME

The bleeding time test

Duke (1910) demonstrated that the duration of bleeding from an ear lobe puncture was dependent on platelet count and independent of coagulation time. Since then modifications as the 'venostasis' introduced by Ivy et al (1941), the 'cuts' introduced by Borchgrevink and Waaler (1958) and lately the use of mechanical disposable devices performing duplicate determinations (Babson and Babson 1978) have greatly improved the sensitivity and reliability of the bleeding time test.

Bleeding time is mainly a measurement of platelet function. At platelet counts below $100 \times 10^9/l$ there is a linear inverse correlation between platelet count and bleeding time and bleeding time is prolonged in patients with impaired platelet function (Marcus 1969, Harker and Slichter 1972a). Patients with haemophilia have normal bleeding time unless their platelet function is depressed (Kaneshiro et al 1969). Anatomical and physiological studies have demonstrated that platelets are primarily involved in the arrest of bleeding from bleeding time wounds (Jørgensen and Borchgrevink 1963, Wester et al 1977, Johnson et al 1979b). In scoliosis the platelet aggregating properties of the patients' collagen has been shown to correlate to the bleeding time (Udén and Nilsson 1979).

Bleeding time after ingestion of acetylsalicylic acid (ASA) has been proposed as a diagnostic aid in haemostatic defects (Quick 1967, Kaneshiro et al 1969). ASA increases bleeding time in both man and experimental animals (Gast 1964, Weiss et al 1968, Evans et al 1968, Buchanan et al 1977). The doses usually used (0.6-2 g) can be expected to inhibit both platelet and endothelial cyclooxygenase (see chapter IV). It is therefore not surprising that the increase in bleeding time upon ASA ingestion is reported to be small and it probably reflects a slight dominance of the TXA_2 effect over the PGI_2 effect. This is in accordance with the finding of a slightly prolonged bleeding time in patients with congenital cyclooxygenase deficiency (Malmsten et al 1975, Pareti et al 1980, Rak and Boda 1980).

Prostacyclin infusion increases bleeding time in rabbits (Ubatuba et al 1979). Amezcua et al (1978) found that a small dose of ASA prolonged bleeding time in rabbits while a large dose did not have this effect. The increase in bleeding time following arachidonate infusion in control rabbits was greatly enhanced by a small dose of ASA while a larger dose abolished this prolonging effect of arachidonate on bleeding time. The changes in bleeding time in these experiments seem to be due to a change in the TXA_2/PGI_2 ratio. In accordance with these results O'Grady and Moncada (1978) were able to demonstrate an increase in bleeding time in 6 human volunteers following 300 mg of ASA while the increase produced by 3.9 g was insignificant. When healthy volunteers were given a single high dose

of ASA the bleeding time remained largely unchanged for the first two hours. Twenty-four and 72 hours later the bleeding time was substantially increased returning to normal levels after 1 week (Amezcuca et al 1979). The TXB₂ generation of the platelets was depressed for the first 72 hours and then increased slowly. These experiments demonstrate that the slower recovery of the platelet cyclooxygenase compared to the recovery of the endothelial cyclooxygenase can be demonstrated by measuring bleeding time.

In the following experiment bleeding time before and after ASA ingestion has been used to investigate if the TXA₂/PGI₂ ratio changes with age in men and also to determine if juvenile diabetics have an altered TXA₂/PGI₂ ratio.

THE PRESENT AUTHORS INVESTIGATIONS (IX, Jørgensen et al. X, Jørgensen et al).

Acetylsalicylic acid, bleeding time and age

(IX, Jørgensen et al).

The problem concerning the dose of ASA which results in the smallest TXA₂/PGI₂ ratio has been dealt with in chapt. IV. The effect of a large dose will, however, depend on the existing TXA₂/PGI₂ ratio. It has also been mentioned that this ratio is altered in atherosclerosis. It would therefore be of great interest to know if the TXA₂/PGI₂ ratio changes with age in normal healthy individuals with no clinical signs of atherosclerosis for which reason the following investigation was carried out. (X, Jørgensen et al).

Ten 11 year old boys from a public school, ten 18-22 and ten 28-32 year old men from drafted military personnel, ten 48-52 year old men from the hospital technical staff and ten 66-70 year old men randomly inquired participated in the investigation. They were all healthy with no history of any chronic disease and no clinical signs of atherosclerosis. Bleeding times were done before and 1½-2 hours after ingestion of different doses of ASA (Idotyl®, Ferrosan, Copenhagen, Denmark, soluble tablets dissolved in water) in the fasting state. The result is given as BT with a subscript indicating the cumulative dose. All determinations in the same volunteer were done within 5 hours. Since plasma concentrations of ASA reach a maximum within

20 minutes after oral administration of an aqueous solution of ASA (Rowland et al 1972) it was assumed that the ASA had reached its maximal effect 1½-2 hours after ingestion. For practical reasons it was impossible to carry out the study with the investigators blinded. At least 2 weeks between each bleeding time determination in each volunteer would have been needed to do this. However, in later investigations (XI, Jørgensen et al) using only one dose of ASA, the medical technicians performing the bleeding time determinations were blinded in respect to ASA/placebo and thereby proved the accuracy and objectivity of the bleeding time determinations.

A preliminary short study had demonstrated that in five men the bleeding time was unchanged when the ASA dose was increased from 1.5 g to 4 g. It was therefore assumed that 25 mg/kg ASA blocked all relevant prostaglandin production. This is in accordance with the results of Masotti et al (1979a) who demonstrated that 8 mg/kg ASA blocked more than 90 % of ischemia induced prostacyclin production in human volunteers.

The main goal of the study was to get an indication of what prostaglandins do to the bleeding time as a function of age. The result was therefore the change in bleeding time upon administration of a large dose of ASA. It was, however, felt that this change in bleeding time had to be related to the 'residual' bleeding time after all relevant prostaglandin production had been blocked. An index Ia was therefore defined as BT_{25}/BT_0 , BT_0 being bleeding time before and BT_{25} bleeding time after ingestion of 25 mg ASA pr kg body-weight. This index will be positive when proaggregatory prostaglandins prevail over antiaggregatory, zero when they balance, and negative when the antiaggregatory prostaglandins prevail. It is therefore an indication of the TXA_2/PGI_2 ratio assuming these are the dominant prostaglandins with respect to bleeding time.

Comparisons of changes within the same volunteer were done utilizing the paired-t-test, comparisons of 2 groups by the student-t-test, while comparisons of more than two groups were done by analysis of variance. A difference was considered significant if $p < 0.05$.

The results of the investigations are shown in table VI and fig. 19. The bleeding time (BT_0) decreased signi-

Table VI. *Bleeding time in men before (BT₀) and after 1 (BT₁), 3.5 (BT_{3.5}) and 25 (BT₂₅) mg ASA/kg.*

Age	BT ₀	BT ₁	BT _{3.5}	BT ₂₅	Ia
11	4.95 (0.39)	8.60 (0.37)	8.35 (0.37)	5.65* (0.28)	0.124 (0.047)
18-22	4.30 (0.32)	6.50 (0.45)	6.65 (0.37)	5.30* (0.40)	0.148 (0.071)
28-32	4.10 (0.19)	5.20 (0.38)	6.30 (0.36)	5.15* (0.47)	0.160 (0.067)
48-52	3.80 (0.23)	not done	6.65 (0.72)	6.30 n.s. (0.69)	0.342 (0.062)
66-70	3.50 (0.19)	4.10 (0.31)	5.50 (0.37)	5.50 n.s. (0.34)	0.350 (0.037)
All	4.13 (0.13)		6.69 (0.25)	5.55 (0.21)	

Results are given as means with SEM in brackets.

*: Significantly different from BT_{3.5}

n.s.: Not significantly different from BT_{3.5}

Ia = BT₂₅-BT₀/BT₂₅.

ificantly with age, while BT₂₅ was independent of age. Ia therefore increased significantly with age. 3.5 mg/kg increased bleeding time significantly in all groups, but only in the younger groups did a further increase in the dose decrease the bleeding time significantly again. The difference between BT₀ and BT₂₅ was also significant in all groups.

The results clearly indicate an inverse correlation between bleeding time and age in the investigated men. This decrease in bleeding time is caused by cyclo-oxygenated arachidonic acid metabolites, since it can be abolished by ASA ingestion. It is therefore interpreted to represent an increase in the TXA₂/PGI₂ ratio with age as demonstrated by the increase in Ia. The results also indicate that differences in dose and age of tested persons may explain conflicting reports on a paradoxical effect of ASA on bleeding time (O'Grady and Moncada 178, Godal et al 1979).

Since it is difficult to know which dose of ASA in a given person prolongs the bleeding time most, it is very difficult to say anything about the TXA₂/PGI₂ ratio from the bleeding time obtained after any standard low

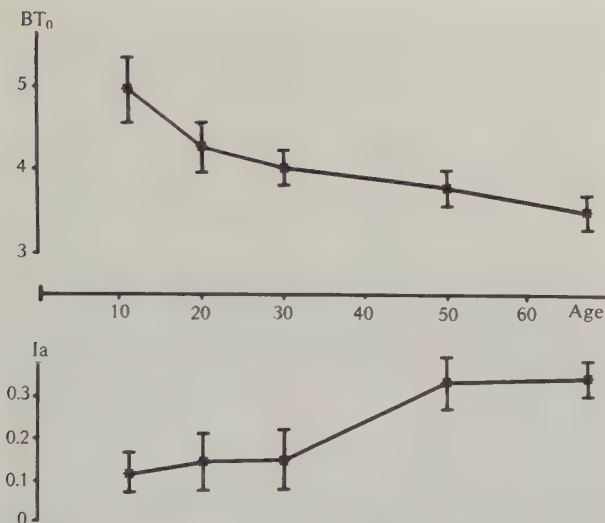


Figure 19. The correlation between bleeding time in minutes (BT_0), the index $Ia = (BT_{25} - BT_0)/BT_{25}$ and age (years) in men. ($\bar{X} \pm S.E.M.$).

ASA dose. However, titration of ASA dose using bleeding time seems logical if ASA is used for antithrombotic purposes. The results clearly demonstrate that a specific TXA_2 inhibitor would be preferable in this context.

When all patients were pooled, the 3.5 mg/kg was the dose which resulted in the longest bleeding time. This is the same dose that Masotti et al (1979a) found inhibited platelet aggregation most while only affecting prostacyclin production to a small degree.

Acetylsalicylic acid and bleeding time in juvenile diabetes mellitus

(X, Jørgensen et al).

As mentioned in chapt. II there are numerous reports indicating platelet hyperaggregability in juvenile diabetes of some duration. Abnormal platelet function in early diabetes is, however, more uncertain. It is therefore unknown if platelets are involved in the genesis of diabetic angiopathy (Hockaday 1979). It would in this respect be of great interest to know if the TXA_2/PGI_2 ratio is altered in early diabetes. Valdorf-Hansen (1967)

found a decreased bleeding time in diabetic patients without clinical signs of angiopathy. This could be an indication of an increased $\text{TXA}_2/\text{PGI}_2$ ratio in early diabetes.

The following study was therefore undertaken to investigate if an altered $\text{TXA}_2/\text{PGI}_2$ ratio could be demonstrated in juvenile diabetics without clinical signs of angiopathy by measurements of bleeding time before and after ASA ingestion.

Twenty young male patients with juvenile diabetes mellitus were investigated. None had clinical signs of diabetic angiopathy. Fifteen patients had bleeding time measured before and after approximately 25 mg/kg ASA, while 5 had the measurement done before and after placebo tablets. The patients received the ASA/placebo in envelopes completely blinded using random numbers. Hemoglobin A_{1c} was determined from dialyzed hemolysates as described by Trivelli et al (1971) as modified by Schwartz et al (1976). Twenty-two age matched controls were investigated before and after ingestion of 25 mg/kg ASA. The bleeding time before ASA/placebo ingestion is termed BT_0 while the bleeding time 2 hours after ingesting ASA/placebo in the fasting state is termed BT_{25} . Statistics were done by the student-t-test and a difference considered significant if $p < 0.05$.

The mean BT_0 in the placebo patients was 4.2 and BT_{25} 4.4. The bleeding time did not change more than 1 minute upon placebo ingestion in any patient. These five patients were used to ensure the objectivity of the measurements. The following results therefore only refer to the 15 patients and 22 controls given ASA. The results are seen in table VII. There was no significant difference in BT_0 between the patients and the controls. However, after ASA ingestion, the 'residual' bleeding time was significantly prolonged in the diabetics. Therefore the diabetics had a significantly increased Ia which is interpreted as an increased $\text{TXA}_2/\text{PGI}_2$ ratio.

We did not confirm the finding of Valdorf-Hansen (1967) of a decreased bleeding time in diabetics, but he investigated a greater number of patients. We did, however, demonstrate a dominance of the proaggregatory prostaglandins over antiaggregatory which was significantly greater in diabetics compared to age and sex matched controls. This finding is in accordance with

Table VII. The distribution of age, hemoglobin A_{1c} (HbA_{1c}), bleeding time (BT₀), bleeding time after ingestion of 25 mg ASA/kg, (BT₂₅) and the index Ia in 15 poorly regulated juvenile diabetics (D) and 22 male controls (C).

	Age	BT ₀	BT ₂₅	BT ₂₅ -BT ₀	Ia	HbA _{1c}
D	21.6 (5.5)	4.9 (0.9)	7.0 (1.3)	2.2 (1.1)	0.302 (0.132)	9.3 (1.4)
C	21.5 (5.3)	4.4 (1.2)	5.3 (1.6)	0.9 (1.6)	0.131 (0.255)	4.3 (0.7)
P	> 0.10	> 0.10	< 0.001	< 0.005	< 0.02	

Results are given as mean with SD in brackets.

Bleeding times are given in minutes.

Hemoglobin A_{1c} is given as % of total hemoglobin concentration.

Ia = (BT₂₅-BT₀)/BT₂₅

indications of a decreased prostacyclin production in diabetes. Harrison et al (1978, 1980) have demonstrated that aorta from streptozocin diabetic rats release reduced amounts of prostacyclin, an abnormality normalized by insulin treatment. Decreased vascular prostacyclin release and decreased levels of 6-keto-PGF_{1α} have been demonstrated in human juvenile diabetes (Johnson et al 1979c, Silberbauer et al 1979c, Dollery et al 1979). Since the increased Ia and hyperaggregable platelets have been demonstrated in early diabetes, it is possible that platelets may be involved in the genesis of diabetic microangiopathy.

FINAL REMARKS

The discovery of thromboxane and prostacyclin has greatly increased our knowledge on the platelet-vessel wall interaction. There are, however, still very many unsolved questions concerning these compounds. Very little is known about the regulation of their production and their 'in vivo' stability. Further their exact role in hemostasis has still not been completely defined.

Research aiming at more effective anti-thrombotic compounds is promising. Thus future research will deal with agents increasing prostacyclin production, stable prostacyclin analogues and thromboxane synthetase inhibitors helping to define the role of thromboxane and prostacyclin in pathological states. A change in the

diet fatty acid composition seems to be another possible pathway of achieving an antithrombotic effect. One of the next steps in this field must be the demonstration of PGI_3 production by vascular microsomes from eicosapentaenoic acid together with answering the question, does eicosapentaenoic acid inhibit endothelial cyclo-oxygenase?

As an adenylate cyclase stimulator prostacyclin may possibly have many actions on the gastro-intestinal tract, renal function, the endocrine, and nervous system. The vast amount of research being carried out in all these fields will beyond any doubt result in gaining of clinically useful knowledge.

RESUMÉ

Introduktion

Når trombocytter stimuleres med en række forskellige stoffer ændrer de form og klumper sammen samtidig med at der secernerer en del biologisk aktive stoffer. Deres reaktivitet er grundlaget for den vigtige rolle trombocytterne spiller i opretholdelsen af karbanens normale integritet. I de senere år har trombocytten imidlertid også tiltrukket sig opmærksomhed som en patogenetisk faktor i flere sygdomsprocesser. Det er derfor af betydelig interesse at forske i de faktorer, der bestemmer trombocytternes reaktivitet. Denne oversigt omhandler balancen mellem to meget potente endogene stoffer, det stærkt pro-aggregatoriske tromboksen og det stærkt anti-aggregatoriske prostacyclin.

Kapitel I

I dette kapitel gives en kort beskrivelse af de anvendte metoder og materiale. Fremstillingen af trombocytfattigt og trombocyttrigt plasma, vaskede trombocyttsuspensioner, præparation af rotte aorta karvæg samt fremstillingen af karvægsmikrosom suspensioner fra kalve aortae og humane navlestrengene gennemgås. Måling af blødningstid og trombocytternes malondialdehyd og tromboksen B_2 produktion beskrives.

Kapitel II

I dette kapitel gennemgås vor viden om trombocytternes rolle i trombose, atherogenese, nyresygdomme og

diabetes mellitus. Trombocytternes vigtige rolle i de arterielle tromboser er formentlig en følge af deres reaktion overfor beskadiget endothel. Deres rolle i atherogenesen og deres betydning ved forskellige nyresygdomme er for nylig blevet gennemgået af forfatteren (I, II, III). Ved diabetes af en vis varighed eller ved diabetes med vaskulære komplikationer er der fundet talrige tegn på forøget trombocytreaktivitet. I de efterfølgende kapitler behandles iagttagelser, der tyder på at tromboksen og prostacyklin spiller en rolle ved disse sygdomme.

Kapitel III

I dette kapitel gennemgås kort opdagelsen af prostaglandiner og det historiske fund af prostaglandin endoperoksiderne. De klassiske prostaglandiners virkning på trombocytter samt trombocytters produktion af klassiske prostaglandiner beskrives kort.

I 1975 isoleredes det meget labile stærkt pro-aggregatoriske tromboksen. Dets eksakte virkningsmekanisme og dets relation til ADP frigørelsen er endnu ikke helt afklaret. I 1976 blev det fundet, at karvægs microsomer omdanner prostaglandin endoperoksider til det labile og stærkt anti-aggregatoriske prostacyklin. Prostacyklins kemiske struktur blev hurtigt fastlagt. Det virker ved at stimulere trombocytens adenylcyklase. Forfatteren har foretaget forsøg, der demonstrerer, at fosfodiesterase hæmmere's anti-aggregatoriske effekt er afhængig af prostacyklin (IV). Endvidere er det vist, at prostacyklin i meget lave koncentrationer kan virke pro-aggregatorisk (V). Prostacyklins stabilitet i plasma er ikke helt klarlagt.

Kapitel IV

I dette kapitel beskrives ændringer i tromboksen og prostacyklin produktionen. Celle-frit plasma indeholder en termolabil, ikke-dialysabel faktor, der stimulerer endothelets prostacyklin produktion. Den uræmiske blødningstendens synes delvist at skyldes øget plasma koncentration af en sådan faktor. I visse tilfælde af det hæmolytiske uræmiske syndrom, spiller arvelig mangel på en sådan faktor muligvis også en rolle (VI).

Frigørelse af arakidonsyre fra cellernes strukturelle membraner er det første led i prostaglandin syntesen. Reguleringen af denne frigørelse er meget dårligt

kendt, men den hæmmes af glucocorticosteroider i både trombocytter og endothelceller (VII). Trombocytter fra patienter med nefrotisk syndrom er hyperreaktive. Dette skyldes formentlig mangel på albumin hæmning af trombocytaggregationen. Albumin hæmmer denne ved at binde arakidonsyre, men det hæmmer også ved en anden ukendt mekanisme (VIII).

Acetylsalicylsyre lammer både tromboksen og prostacyklin syntesen ved at hæmme cyklooksygenasen. Imidlertid synes trombocytten mere følsom end endothelcellen, hvilket muliggør en overvejende lammelse af tromboksen produktionen. Mere effektivt i anti-trombotisk øjemed ville en specifik tromboksen syntetase hæmmer være. En del lipooksygenerede arakidonsyre metabolitter hæmmer prostacyklin syntetasen. Dette er muligvis forklaringen på den nedsatte prostacyklin produktion ved atherosklerose og E-vitamin mangel.

Skønt virkningsmekanismen endnu ikke er helt klarlagt kan balancen mellem anti- og pro-aggregatoriske prostaglandiner forskydes i anti-trombotisk retning ved at øge kostens indhold af eikosapentaensyre.

Kapitel V

I dette kapitel præsenteres en række forsøg, der viser, at stykker af rotte karvæg kan anvende endoperoksider produceret i trombocytter til prostacyklin produktion. Ligeså kan trombocytter anvende endoperoksider produceret i karvæggen til tromboksen produktion. Denne udveksling af endoperoksider er imidlertid formentlig minimal og har næppe nogen særlig betydning under normale omstændigheder.

Kapitel VI

I dette kapitel gennemgås først tilgængelige data, der viser at blødningstidsbestemmelser før og efter indgift af acetylsalicylsyre kan bruges til at vurdere 'in vivo' balancen mellem tromboksen og prostacyklin. Herefter fremlægges resultater, der tyder på, at tromboksen/prostacyklin forholdet stiger med alderen (IX), samt at det er forøget hos juvenile diabetikere (X).

References:

- Amezcuca JL, O'Grady J, Salmon JA, Moncada S: Prolonged paradoxical effect of aspirin on platelet behaviour and bleeding time in man. *Thromb Res* 1979; 16: 69-79.
- Amezcuca JL, Parsons M, Moncada S.: Unstable metabolites of arachidonic acid and the formation of the haemostatic plug. *Thromb Res* 1978; 13: 477-88.
- AMIS — Aspirin Myocardial Infarction Study Research Group: A randomised controlled trial of aspirin in persons recovered from myocardial infarction. *JAMA* 1980; 243: 661-9.
- Annual Report from the Chief Medical Officer in Greenland. 1963-67, 1973-76.
- Anon.: Prostaglandins 1977; 13: 375.
- Apitz K.: Ueber Profibrin. IV. Die Agglutination von Blutplättchen durch Profibrin. *Z. Gesamte Exp Med* 1939; 105: 89-94.
- Ardlie NG, Glew G, Schultz BG, Schwartz CJ: Inhibition and reversal of platelet aggregation by methylxanthines. *Thromb Diath Haemorrh* 1967; 18: 670-3.
- Ashford TP, Freiman DG: The role of the endothelium in the initial phases of thrombosis: An electron microscopic study. *Amer J Path* 1967; 50: 257-66.
- Ashida S, Sakuma K, Abiko Y: Antithrombotic effects of ticlopidine, acetylsalicylic acid and dipyridamole in vascular shunt model in rats. *Thromb Res* 1980; 17: 663-71.
- Babson SR, Babson AL: Development and evaluation of a disposable device for performing simultaneous duplicate bleeding time determinations. *Am J Clin Path* 1978; 70: 406-8.
- Baenziger NL, Becherer PR, Majerus PW: Characterization of prostacyclin synthesis in cultured human arterial smooth muscle cells, venous endothelial cells and skin fibroblasts. *Cell* 1979; 16: 967-74.
- Baenziger NL, Dillender MJ, Majerus PW: Cultured human skin fibroblasts and arterial cells produce a labile platelet-inhibitory prostaglandin. *Biochem Biophys Res Comm* 1977; 78: 294-301.
- Bang HO, Dyerberg J, Hjørne N: The composition of food consumed by Greenland Eskimos. *Acta Med Scand* 1976; 200: 69-73.
- Bang NU, Trygstad CW, Schroeder JE, Heidenreich RD, Csicsko BM: Enhanced platelet function in glomerular renal disease. *J Lab Clin Med* 1973, 81: 651-60.
- Barden TP: Induction of labor with prostaglandins. In: Ramwell PW, ed. *Prostaglandins*, Vol 3, Plenum Press, New York, 1977: pp. 109-33.
- Basista M, Dobranowski J, Gryglewski RJ: Prostacyclin and thromboxane generating systems in rabbits pretreated with aspirin. *Pharmacol Res Commun* 1978; 10: 759-63.

- Baumgartner HR: Platelet interaction with vascular structures. *Thromb Diath Haemorrh* 1972; 51 (suppl.), 161-75.
- Baumgartner HR: The role of blood flow in platelet adhesion, fibrin deposition, and formation of mural thrombi. *Microvascular Research* 1973; 5: 167-79.
- Baumgartner HR: Morphometric quantitation of adherence of platelets to an artificial surface and components of connective tissue. *Thromb Diath Haemorrh* 1974; 60: (suppl.), 39-49.
- Baumgartner HR, Muggli R, Tschopp TB, Turitto VT: Platelet adhesion, release and aggregation in flowing blood: effects of surface properties and platelet function. *Thrombos Haemostas* 1976; 35: 124-38.
- Baumgartner HR, Stemerman MB, Spaet TH: Adhesion of blood platelets to subendothelial surface: Distinct from adhesion to collagen. *Experienta* 1971; 27: 283-5.
- Bayer B-L, Blass K-E, Förster W: Anti-aggregatory effect of prostacyclin (PGI₂) in vivo. *Br J Pharmac* 1979; 66: 10-2.
- Bensoussan D, Levy-Toledano S, Passa P, Caen J, Canivet J: Platelet hyperaggregation and increased plasma level of von Willebrand factor in diabetics with retinopathy. *Diabetologia* 1975; 11: 307-12.
- Bergström S, Danielsson H, Samuelsson B: The enzymatic formation of prostaglandin E₂ from arachidonic acid. Prostaglandins and related factors 32. *Biochim Biophys Acta* 1964; 90: 207-10.
- Bergström S, Ryhage R, Samuelsson B, Sjövall J: The structures of prostaglandin E₁, F_{1α}, F_{1β}. *J Biol Chem* 1963; 238: 3555-64.
- Berlyne BM, Mallick NP: Ischemic heart-disease as a complication of nephrotic syndrome. *Lancet* 1969; II: 399-400.
- Berridge MJ: The interaction of cyclic nucleotides and calcium in the control of cellular activity. *Adv Cyclic Nucleotide Res* 1975; 6: 1-98.
- Best LC, Martin TJ, Russel RGG, Preston FE: Prostacyclin increases cyclic AMP levels and adenylate cyclase activity in platelets. *Nature* 1977; 267: 850-2.
- Bigelow FS: Serotonin activity in blood: Measurements in normal subjects and in patients with thrombocythemia, hemorrhagica, and other hemorrhagic states. *J Lab Clin Med* 1954; 43: 759-73.
- Bills TK, Smith JB, Silver MJ: Metabolism of ¹⁴C-arachidonic acid by human platelets. *Biochim Biophys Acta* 1976; 424: 303-14.
- Bills TK, Smith J, Silver MJ: Selective release of arachidonic acid from the phospholipids of human platelets in response to thrombin. *J Clin Invest* 1977; 60: 1-6.
- Bills TK, Smith JB, Silver MJ: Intracellular regulation of the metabolism of arachidonic acid in human platelets. *Thromb Haemostas* 1978; 40: 219-23.

- Bizzozzero J: Ueber einen neuen Formbestandteil des Blutes und dessen Rolle bei Thrombose und der Blutgerinnung. *Virchows Arch Pathol Anat Physiol Klin Med* 1882; 90: 261-332.
- Black KL, Culp B, Madison D, Randall OS, Lands WEM: The protective effects of dietary fish oil on focal cerebral infarction. *Prostaglandins and Medicine* 1979; 3: 257-68.
- Blackwell GJ, Duncombe WG, Flower RJ, Parsons MF, Vane JR: The distribution and metabolism of arachidonic acid in rabbit platelets during aggregation and its modification by drugs. *Br J Pharmac* 1977, 59: 353-66.
- Blajchman MA, Senyi AF, Hirsh J, Surya Y, Buchanan M, Mustard MA: Shortening of the bleeding time in rabbits by hydrocortisone caused by inhibition of prostacyclin generation by the vessel wall. *J Clin Invest* 1979; 63: 1026-35.
- Blumberg AL, Denny SE, Marshall GR, Needleman P: Blood vessel-hormone interactions: angiotensin, bradykinin and prostaglandins. *Am J Physiol* 1977; 232: H305-H10.
- Borchgrevink CF, Waaler BA: The secondary bleeding time. A new method for differentiation of hemorrhagic diseases. *Acta Med Scand* 1958; 162: 361-74.
- Borda ES, Lazzari MA, Gimeno MF, Gimeno AL: Human platelet rich plasma and human serum protects from inactivation the antiaggregatory capacity of prostacyclin-like material (PGI₂) produced by rat stomach fundus. *Prostaglandins* 1980; 19: 899-905.
- Born GVR: Aggregation of blood platelets by adenosine diphosphate and its reversal. *Nature* 1962; 194: 927-9.
- Born GVR: Fluid-mechanical and biochemical interactions in haemostasis. *Br Med Bull* 1977; 33: 193-7.
- Born GVR, Cross MJ: Effect of adenosine diphosphate on the concentration of platelets in circulating blood. *Nature* 1963, 197: 974-6.
- Borsey DQ, Dawes J, Fraser DM, Prowse CV, Elton RA, Clarke BF: Plasma beta-thromboglobulin in diabetes mellitus. *Diabetologia* 1980; 18: 353-7.
- Bounameaux Y, Roskam J: L'accolement des plaquettes aux fibres sous-endotheliales. *CR Seances' Soc Biol* 1959; 153: 865-7.
- Bourgain RH: The inhibitory effect of PGI₂ (prostacyclin) on white platelet arterial thrombus formation. *Haemostasis* 1979; 8: 117-9.
- Bourgain RH: The inhibition of PGI₂ synthetase within the arterial wall by 15-hydroperoxyarachidonic acid enhances local white platelet thrombosis. *Haemostasis* 1980; 9: 345-51.
- Bourgain RH, Andries R, Finne E: The role of prostaglandins in platelet-vessel wall interaction. *Arch Int Pharmacodyn* 1979; 239: 161-3.

- Breiddin K, Grun H, Krzywanek HJ, Schremmer WP: On the measurement of spontaneous platelet aggregation: The platelet aggregation test III. Methods and first clinical results. *Thromb Haemostas* 1976; 35: 669-91.
- le Breton GC, Venton DL, Enke SE, Halushka PV: 13-azaprostanoic acid: A specific antagonist of the human blood platelet thromboxane/endoperoxide receptor. *Proc Natl Acad Sci (USA)* 1979; 76: 4097-101.
- Buchanan MR, Dejana E, Cazenave J-P, Mustard JF, Hirsh J: Uncontrolled PGI₂ production by whole vessel wall segments due to thrombin generation in vivo and its prevention by heparin. *Thromb Res* 1979, 16: 551-5.
- Buchanan MR, Hirsh J: A comparison of the effects of aspirin and dipyridamole on platelet aggregation in vivo and ex vivo. *Thromb Res* 1978; 13: 517-29.
- Buchanan GR, Martin V, Levine PH, Scoon K, Handin RI: The effects of »anti-platelet« drugs on bleeding time and platelet aggregation in normal human subjects. *Am J Clin Path* 1977; 68: 355-9.
- Bunting S, Gryglewski R, Moncada S, Vane JR: Arterial walls generate from prostaglandin endoperoxides a substance (prostaglandin X) which relaxes strips of mesenteric and coeliac arteries and inhibits platelet aggregation. *Prostaglandins* 1976b; 12: 897-912.
- Bunting S, Moncada S, Vane JR: The effects of prostaglandin endoperoxides and thromboxane A₂ on strips of rabbit coeliac artery and certain other smooth muscle preparations. *Brit J Pharmacol Chem* 1976a; 57: pp. 462-3.
- Bunting S, Moncada S, Vane JR: Antithrombotic properties of vascular endothelium. *Lancet* 1977; II: 1075-6.
- Burch JW, Baenziger NL, Stanford N, Majerus PW: Sensitivity of fatty acid cyclooxygenase from human aorta to acetylation by aspirin. *Proc Natl Acad Sci (USA)* 1978b; 75: 5181-4.
- Burch JW, Stanford N, Majerus PW: Inhibition of platelet prostaglandin synthetase by oral aspirin. *J Clin Invest* 1978a; 61: 314-9.
- Busch C, Wasteson Å, Westermark B: Release of a cell growth promoting factor from human platelets. *Thromb Res* 1976; 8: 493-500.
- Bye A, Lewis Y, O'Grady J: Effect of a single oral dose of aspirin on the platelet aggregation response to arachidonic acid. *Br J Clin Pharmacol* 1979; 7: 283-6.
- Cameron JS: Platelets and Glomerulonephritis. *Nephron* 1977; 18: 253-8.
- Cazenave J-P, Davies JA, Senyi AF, Blajchman MA, Hirsch J, Mustard JF: Effect of methylprednisolone on platelet adhesion to damaged aorta bleeding time and platelet survival. *Blood* 1976; 48: 1009.

- Cazenave J-P, Dejana E, Kinlough-Ratbone RL, Richardson M, Packham MA, Mustard JF: Prostaglandins I_2 and E_2 reduce rabbit and human platelet adherence without inhibiting release from adherent platelets. *Thromb Res* 1979; 15: 273-9.
- Cerkus AL, Ali M, Davies BJ, McDonald JWD: Possible significance of small numbers of functional platelets in a population of aspirin-treated platelets in vivo and in vitro. *Thromb Res* 1980; 18: 389-97.
- Chang W-C, Nakao J, Orimo H, Murota S-I: Stimulation of prostacyclin biosynthetic activity by estradiol in rat aortic smooth muscle cells in culture. *Biochim Biophys Acta* 1980; 619: 107-18.
- Chapman I: Morphogenesis of occluding coronary artery thrombosis. *Arch Pathol* 1965; 80: 256-361.
- Charo IF, Feinman RD, Detwiler TC: Interrelations of Platelet Aggregation and Secretion. *J Clin Invest* 1977; 60: 866-73.
- Charo IF, Feinman RD, Detwiler TC, Smith JB, Ingberman CM, Silver MJ: Prostaglandin endoperoxides and thromboxane A_2 can induce platelet aggregation in the absence of secretion. *Nature* 1977; 269: 66-9.
- Cho MJ, Allen MA: Chemical stability of prostacyclin (PGI_2) in aqueous solution. *Prostaglandins* 1978; 15: 943-54.
- Claesson HE, Malmsten C: On the interrelationship of prostaglandin endoperoxide G_2 and cyclic nucleotides in platelet function. *Eur J Biochem* 1977; 76: 277-84.
- Clark WF, Friesen M, Linton AL, Lindsay RM: The platelet as a mediator of tissue damage in immune complex glomerulonephritis. *Clin Nephrol* 1976; 6: 287-9.
- Clarkson AR, Seymour AE, Jackson B: Coagulation and renal disease. *Med J Aust* 1976; 1: 573-8.
- Clausen J, Srivastava KC: The synthesis of prostaglandins in human platelets. *Lipids* 1972; 7: 246-50.
- Coller BS, Frank RN, Milton RC, Grallnick HR: Plasma cofactors of platelet function: Correlation with diabetic retinopathy and hemoglobins A_{1a-c} . *Ann Int Med* 1978; 88: 311-6.
- Collier HOJ: New light on how aspirin works. *Nature* 1969; 223: 35-7.
- Constantine JW: Aggregation of guinea-pig platelets by adenosine diphosphate. *Nature* 1966; 210: 162-4.
- Constantinides P: Plaque fissures in human coronary thrombosis. *J Atheroscler Res* 1966; 6: 1-17.
- Corey EJ, Keck GE, Székely I: Synthesis of Vane's prostaglandin X; 6,9 α -Oxido-9 α , 15 α -dihydroxyprosta-(Z) 5, (E) 13-dienoic acid. *J Am Chem Soc* 1977; 99: 2006-8.
- Corey EJ, Nicolaou KC, Machida Y, Malmsten CL, Samuelsson B: Synthesis and biological properties of a 9,11-azoprostanoid: Highly active biochemical mimic of prostaglan-

- din endoperoxides. *Proc Natl Acad Sci (USA)* 1975; 72: 3355-8.
- Craven LL: Experiences with aspirin (acetylsalicylic acid) in the nonspecific prophylaxis of coronary thrombosis. *Mississippi Valley Medical Journal* 1953; 75: 38-44.
- Culp BR, Titus BG, Lands WEM: Inhibition of prostaglandin biosynthesis by eicosapentaenoic acid. *Prostaglandins and Medicine* 1979; 3: 269-78.
- Czervionke RL, Hoak JC, Fry GL: Effect of aspirin on the thrombin-induced adherence of platelets to cultured cells from blood vessel wall. *J Clin Invest* 1978; 62: 847-56.
- Dam H: Influence of antioxidants and redox substances on signs of vitamin E deficiency. *Pharm Rev* 1957; 9: 1-16.
- D'Angelo V, Villa S, Mysliwiec M, Donati MB, de Gaetamp G: Defective fibrinolytic and prostacyclin-like activity in human atheromatous plaques. *Thrombos Haemostas* 1978; 39: 535-6.
- Danon A, Assouline G: Inhibition of prostaglandin biosynthesis by corticosteroids requires RNA and protein synthesis. *Nature* 1978; 273: 552-4.
- Day HJ, Holmsen H: Concepts of the blood platelet release reaction. *Ser Haematol* 1971; 4: 3-27.
- Dembinska-Kiec A, Gryglewska T, Zmuda A, Gryglewski RJ: The generation of prostacyclin by arteries and by the coronary vascular bed is reduced in experimental atherosclerosis in rabbits. *Prostaglandins* 1977; 14: 1025-35.
- Dembinska-Kiec A, Rücker W, Schönhöfer PS: Atherosclerosis decreased prostacyclin formation in rabbit lungs and kidneys. *Prostaglandins* 1979a; 17: 831-8.
- Dembinska-Kiec A, Rücker W, Schönhöfer PS: Prostacyclin-dependent differences in TXA₂ formation by platelets from normal and atherosclerotic rabbits. *Atherosclerosis* 1979b; 33: 217-26.
- Derksen A, Cohen P: Pattern of fatty acid release from endogenous substrates by human platelet homogenates and membranes. *J Biol Chem* 1975; 250: 9342-7.
- Detwiler TC, Charo IF, Feinman RD: Evidence that calcium regulates platelet function. *Thrombos Haemostas* 1978; 40: 207-11.
- Diczfalusy U, Hammarström S: Inhibitors of thromboxane synthetase in human platelets. *FEBS Letters* 1977; 82: 107-110.
- Di Rosa M, Persico P: Mechanism of inhibition of prostaglandin biosynthesis by hydrocortisone in rat leucocytes. *Br J Pharmac* 1979; 66: 161-3.
- Dollery CT, Friedman LA, Hensby CN, Kohner E, Lewis PJ, Porta M, Webster J: Circulating prostacyclin may be reduced in diabetes. *Lancet* 1979; II: 1365.

- van Dorp DA, Beerthuis RK, Nugteren DM, Vonkeman H: The biosynthesis of prostaglandins. *Biochim Biophys Acta* 1964; 90: 204-7.
- van Dorp DA, Buytenhek M, Christ-Hazelhof E, Nugteren DH, van der Ouderaa FJ: Isolation and properties of enzymes involved in prostaglandin biosynthesis. *Acta Biol Med Germ* 1978; 37: 691-9.
- Duke WW: The relation of blood platelets to hemorrhagic disease. *JAMA* 1910; 555: 1185-92.
- Durham RCH: A unified theory of the control of actin and myosin in non-muscle movements. *Cell* 1974, 2: 123-35.
- Dusting GJ, Moncada S, Vane JR: Recirculation of prostacyclin (PGI_2) in the dog. *Br J Pharm* 1978; 64: 315-20.
- Dyerberg J, Bang HO: Dietary fat and thrombosis. *Lancet* 1978; I: 152.
- Dyerberg J, Bang HO: Lipid metabolism, atherogenesis, and haemostasis in eskimos: the role of the prostaglandin-3 family. *Haemostasis* 1979a; 8: 227-33.
- Dyerberg J, Bang HO: Hæmostatic function and platelet polyunsaturated fatty acids in eskimos. *Lancet* 1979b; II: 433-5.
- Dyerberg J, Bang HO: Et forslag til thrombosaproylakse. Eskimo-modellen, *Ugeskr Læger* 1980; 142: 1597-600.
- Dyerberg J, Bang HO, Hjørne H: Fatty acid composition of the plasma lipid in Greenland Eskimos. *Am J Clin Nutr* 1975; 28: 958-66.
- Dyerberg J, Bang HO, Stoffersen E, Moncada S, Vane JR: Eicosapentaenoic acid and prevention of thrombosis and atherosclerosis? *Lancet* 1978; II: 117-9.
- Eberth JC, Schimmelbusch C: Experimentelle Untersuchungen über Thrombose. *Virchows Arch Pathol Anat Physiol Klin Med* 1886; 103: 39-87.
- Elwood PC, Cochrane AL, Burr ML, Sweetnam PM, Williams G, Welsby E, Hughes SJ, Renton R: A randomized controlled trial of acetyl salicylic acid in the secondary prevention of mortality from myocardial infarction. *Brit Med J* 1974; 1: 436-40.
- Elwood PC, Sweetnam PM: Aspirin and secondary mortality after myocardial infarction. *Lancet* 1979; II: 1313-5.
- Emmons PR, Hampton JR, Harrison MJG, Honour AJ, Mitchell JRA: Effect of prostaglandin E_1 on platelet behavior in vitro and in vivo. *Brit Med J* 1967; 22: 468-72.
- Emmons PR, Harrison MJG, Honour AJ, Mitchell JRA: Effect of dipyridamole on human platelet behaviour. *Lancet* 1965; II: 603-6.
- von Euler US: On the specific vaso-dilating and plain muscle stimulating substances from accessory genital glands in man and certain animals (prostaglandin and vesiglandin). *J Physiol (London)* 1936; 88: 213-34.

- Evans G, Packham MA, Nishizawa EE, Mustard JF, Murphy EA: The effect of acetylsalicylic acid on platelet function. *J Exp Med* 1968; 128: 877-94.
- Feinman RD, Detwiler TC: Platelet secretion induced by divalent cation ionophores. *Nature* 1974; 249: 172-3.
- Ferreira SH, Vane JR: Prostaglandins: Their disappearance form and release into the circulation. *Nature* 1967; 216: 868-73.
- Filipovic I, Rutemöller M: Comparative studies on fatty acid synthesis fatty acid synthesis in atherosclerotic and hypoxic human aorta. *Atherosclerosis* 1976; 24: 457-69.
- Fitzpatrick FA, Bundy GL, Gorman RR, Honohan T: 9,11-epoxyiminoprostanoic acid is a thromboxane A_2 antagonist in human platelets. *Nature* 1978; 275: 764-6.
- Fitzpatrick F, Gorman R, Bundy G, Honohan T, McGuire J, Sun F: 9,11, iminoepoxyprosta-5,13-dienoic acid is a selective thromboxane A_2 synthetase inhibitor. *Biochim Biophys Acta* 1979; 673: 238-44.
- Flack JD: The hypothalamus-pituitary-endocrine system. In: Ramwell PW, ed. *Prostaglandins*, Vol. 1, Plenum Press, New York, 1973, pp. 327-45.
- Flamenbaum W, Kleinman JG: Prostaglandins and renal function or »a trip down the rabbit hole«. In: Ramwell PW, ed. *Prostaglandins*, Vol. 3, Plenum Press, New York, 1977: pp. 267-328.
- Flower RJ, Blackwell JG: The importance of phospholipase- A_2 in prostaglandin biosynthesis. *Biochem Pharmacol* 1976; 25: 285-91.
- French JE, MacFarlane RG, Sanders AG: The structure of haemostatic plugs and experimental thrombi in small arteries. *Brit J Exp Path* 1964; 45: 467-74.
- Fried J, Barton J: Synthesis of 13,14-dehydroprostacyclin methyl ester: a potent inhibitor of platelet aggregation. *Proc Natl Acad Sci (USA)* 1977; 74: 2199-203.
- Fuster V, Bowie EJW, Lewis JC, Fass DN, Owen CA jr, Brown AL: Resistance to arteriosclerosis in pigs with von Willebrand's disease. *J Clin Invest* 1978; 61: 722-30.
- Gaarder A, Jonsen J, Laland S, Hellem A, Owren PA: Adenosine diphosphate in red cells as a factor in the adhesiveness of human blood platelets. *Nature* 1961; 192: 531-2.
- Gast LF: Influence of aspirin on haemostatic parameters. *Ann Rheum Dis* 1964; 23: 500-4.
- George CRP, Slichter SJ, Quadracci LJ, Striker GE, Harker LA: A kinetic evaluation of hemostasis in renal disease. *New Engl J Med* 1974; 291: 1111-5.
- Gerrard JM, Peller JD, Krick TP, White JG: Cyclic AMP and platelet prostaglandin synthesis. *Prostaglandins* 1977; 14: 39-50.

- Gerrard J, Townsend D, Stoddard S, Witkop CJ, White JG: The influence of prostaglandin G_2 on platelet ultrastructure and platelet secretion. *Am J Path* 1977; 86: 99-110.
- Gerrard JM, White JG, Peterson DA: The platelet dense tubular system: its relationship to prostaglandin synthesis and calcium flux. *Thrombos Haemostas* 1978; 40: 224-31.
- Gimeno MF, Sterin-Borde L, Borde ES, Lazzari MA, Gimeno AL: Human plasma transforms prostacyclin (PGI_2) into a platelet antiaggregatory substance which contracts isolated arteries. *Prostaglandins* 1980; 19: 907-15.
- Glavind J, Hartman S, Clemensen J, Jessen KE, Dam H: Studies on the role of lipoperoxides in human pathology. II. The presence of peroxidized lipids in the atherosclerotic aorta. *Acta Path Microbiol Scand* 1952; 30: 1-6.
- Godal HC, Eika C, Dybdahl JH, Daae L, Larsen S: Aspirin and bleeding time. *Lancet* 1979; I: 1236.
- Goodman DS: The interaction of human serum albumin with long chain fatty acid anions. *J Am Chem Soc* 1958; 80: 3892-8.
- Gorman RR, Bunting S, Miller OV: Modulation of human platelet adenylate cyclase by prostacyclin (PGX) Prostaglandins 1977b; 13: 377-88.
- Gorman RR, Buntly BL, Peterson DC, Sun FF, Miller OV, Fitzpatrick FA: Inhibition of human platelet thromboxane synthetase by 9,11-azoprostano-5,13-dienoic acid. *Proc Natl Acad Sci (US)* 1977a; 74: 4007-11.
- Gorman RR, Fitzpatrick FA, Miller OV: Reciprocal regulation of human platelet cAMP levels by thromboxane A_2 and prostacyclin. In: George WJ, Ignarro LJ, eds. *Advances in cyclic nucleotide research*. Raven Press, New York, 1978; pp. 597-609.
- Gorman RR, Hopkins NK: Agonist-specific desensitization of PGI_2 -stimulated cyclic AMP accumulation by PGE_1 in human foreskin fibroblasts. *Prostaglandins* 1980; 19: 2-16.
- Grette K: The mechanism of thrombin-catalyzed hemostatic reactions in platelets. *Acta Physiol Scand* 1962; 195: (suppl.), 1-93.
- Grodzinska L, Marcinkiewicz E: The generation of TXA_2 in human platelet rich plasma and its inhibition by nictindole and prostacyclin. *Pharmacol Res Commun* 1979; 11: 133-46.
- Gryglewski RJ, Bunting S, Moncada S, Flower RJ, Vane JR: Arterial walls are protected against deposition of platelet thrombi by a substance (prostaglandin X) which they make from prostaglandin endoperoxides. *Prostaglandins* 1976; 12: 685-713.
- Gryglewski RJ, Dembinska-Kiec A, Zmuda A, Gryglewska T: Prostacyclin and thromboxane A_2 biosynthesis capacities of heart arteries and platelets at various stages of experimental atherosclerosis in rabbits. *Atherosclerosis* 1978a; 31: 385-94.

- Gryglewski RJ, Korbut R, Ocetkiewicz A: Deaggregatory action of prostacyclin in vivo and its enhancement by theophylline. *Prostaglandins* 1978b; 15: 637-49.
- Gryglewski RJ, Korbut R, Ocetkiewicz A, Splawinski J, Wotaszek B, Swies J: Lungs as a generator of prostacyclin — Hypothesis on physiological significance. *Naunyn-Schmiedeberg's Arch Pharmacol* 1978c; 304: 45-50.
- Gryglewski RJ, Panczenko B, Korbut R, Grodzinska L, Ocetkiewicz A: Corticosteroids inhibit prostaglandin release from perfused mesenteric blood vessels of rabbit and from perfused lungs of sensitized guinea pig. *Prostaglandins* 1975; 10: 343-55.
- Gryglewski RJ, Salmon JA, Ubatuba FB, Weatherby BC, Moncada S, Vane JR.: Effects of all, cis 5, 8, 11, 14, 17 eicosapentaenoic acid and PGH₂ on platelet aggregation. *Prostaglandins* 1979; 18: 453-78.
- Gryglewski RJ, Szczeklik A, Nizankowski R: Anti-platelet action of intravenous infusion of prostacyclin in man. *Thromb Res* 1978d; 13: 153-63.
- Gryglewski RJ, Zmuda A, Korbut R, Krecioch E, Bieron K: Selective inhibition of thromboxane A₂ biosynthesis in blood platelets. *Nature* 1977; 267: 627-8.
- Hagenfeldt L, Wahren J: Turnover of plasma-free arachidonic and oleic acid in resting and exercising human subjects. *Metabolism* 1975; 24: 799-806.
- Ham EA, Egan RW, Soderman DD, Gale PH, Kuehl FA: Peroxidase-dependent deactivation of prostacyclin synthetase. *J Biol Chem* 1979; 254: 2191-4.
- Hamberg M: Transformation of 5, 8, 11, 14, 17-eicosapentaenoic acid in human platelets. *Biochim Biophys Acta* 1980; 618: 389-98.
- Hamberg M, Samuelsson B: On the metabolism of prostaglandin E₁ and E₂ in man. *J Biol Chem* 1971; 246: 6713-21.
- Hamberg M, Samuelsson B: Detection and isolation of an endoperoxide intermediate in prostaglandin biosynthesis. *Proc Natl Acad Sci (USA)* 1973; 70: 899-903.
- Hamberg M, Samuelsson B: Prostaglandin endoperoxides. Novel transformation of arachidonic acid in human platelets. *Proc Natl Acad Sci (USA)* 1974; 71: 3400-4.
- Hamberg M, Svensson J, Samuelsson B: Prostaglandin endoperoxides. A new concept concerning the mode of action and release of prostaglandins. *Proc Natl Acad Sci (USA)* 1974b; 71: 3824-8.
- Hamberg M, Svensson J, Samuelsson B. Thromboxanes: A new group of biologically active compounds derived from prostaglandin endoperoxides. *Proc Natl Acad Sci (USA)* 1975; 72: 2994-8.
- Hamberg M, Svensson J, Wakabayashi T, Samuelsson B. Isolation and structure of two prostaglandin endoperoxi-

- des that cause platelet aggregation. *Proc Natl Acad Sci (USA)* 1974 a; 71: 345-9.
- Harker LA, Ross R, Slichter SJ, Scott CR. Homocystine-induced arteriosclerosis: The role of endothelial cell injury and platelet response in its genesis. *J Clin Invest* 1976; 58: 731-41.
- Harker LA, Slichter SJ. The bleeding time as a screening test for evaluation of platelet function. *New Engl J Med* 1972a; 287: 155-9.
- Harker LA, Slichter SJ. Platelet and fibrinogen consumption in man. *New Engl J Med* 1972b; 287: 999-1005.
- Harker LA, Slichter SJ. Arterial and venous thrombo-embolism: Kinetic characterization and evaluation of therapy. *Thromb Diath Haemorrh* 1974; 31: 188-203.
- Harman D. Free radical theory of aging. *Triangle* 1973; 12: 153-6.
- Harrison HE, Reece AH, Johnson M. Decreased vascular prostacyclin in experimental diabetes. *Life Sciences* 1978; 23: 351-6.
- Harrison HE, Reece AH, Johnson M. Effect of insulin treatment on prostacyclin in experimental diabetes. *Diabetologia* 1980; 18: 65-8.
- Harter HR, Burch JW, Majerus PW, Stanford N, Delmez JA, Anderson CB, Weerts CA. Prevention of thrombosis in patients on hemodialysis by low-dose aspirin. *New Engl J Med* 1979; 301: 577-9.
- Harvald B. Mødereferat: Third international symposium on circumpolar health. Yellowknife, NWT Canada 1974. *Ugeskr Læger* 1974; 136: 2461-2.
- Haslam RJ. Role of adenosine diphosphate in the aggregation of human blood-platelets by thrombin and by fatty acids. *Nature* 1964; 202: 765-8.
- Haslam RJ: Interactions of the pharmacological receptors of blood platelets with adenylate cyclase. *Ser Haematol* 1973; 6: 333-50.
- Haslam RJ, Davidson MML, Fox JEB, Lynham JA. Cyclic nucleotides in platelet function. *Thrombos Haemostas* 1978; 40: 232-40.
- Heath H, Brigden WD, Canever JV, Pollock J, Hunter PR, Kelsey J, Bloom A. Platelet adhesiveness and aggregation in relation to diabetic retinopathy. *Diabetologia* 1971; 7: 308-15.
- Helmer M, Lands WEM, Smith WL. Purification of the cyclooxygenase that forms prostaglandins? Demonstration of two forms of iron in the holoenzyme. *J Biol Chem* 1976; 251: 5575-9.
- Hensby CN, Dollery CT, Barnes PJ, Dargie H. Production of 6-oxo-PGF_{1α} by human in vivo. *Lancet* 1979; II: 1162-3.

- Herman AG, Moncada S, Vane JR. Formation of prostacyclin (PGI₂) by different layers of the arterial wall. *Arch Int Pharmacodyn* 1977; 227: 162-3.
- Ho PKK, Hermann RG, Towner RD, Walters CP. Reversal of platelet aggregation by aortic microsomes. *Biochem Biophys Res Comm* 1977; 74: 514-9.
- Hockaday TDR. Direct animal and indirect human evidence of altered platelet function in diabetes. *Metabolism* 1979; 28: 415-22.
- Holmsen H, Day HJ, Stormorken H. The blood platelet release reaction. *Scand J Haemat* 1969; 8: 3-26.
- Hong SL. Effect of bradykinin and thrombin on prostacyclin synthesis in endothelial cells from calf and pig aorta and human umbilical vein. *Thromb Res* 1980; 18: 787-95.
- Hong SL, Levine L. Inhibition of arachidonic acid release from cells as the biochemical action of anti-inflammatory corticosteroids. *Proc Natl Acad Sci (USA)* 1976; 73: 1730-4.
- Honour AJ, Mitchell JRA. Platelet clumping in vivo. *Nature* 1967; 197: 1019-20.
- Hope W, Martin TJ, Chesterman CN, Morgan FJ. Human β -thromboglobulin inhibits PGI₂ production and binds to a specific site in bovine aortic endothelial cells. *Nature* 1979; 282: 210-2.
- Hornstra G, Haddeman E, Don JA. Some investigations into the role of prostacyclin in thromboregulation. *Thromb Res* 1978; 12: 367-74.
- Hornstra G, Haddeman E, Don JA. Blood platelets do not provide endoperoxides for vascular prostacyclin production. *Nature* 1979; 279: 66-8.
- Hornstra G, Hemker HC. Clot promoting effect of platelet-vessel wall interaction: Influence of dietary fats and relation to arterial thrombus formation in rats. *Haemostasis* 1979; 8: 211-26.
- Hovig T. The ultrastructure of rabbit blood platelet aggregates. *Thromb Diath Haemorrh* 1962; 8: 455-71.
- Hovig T, Rowsell HC, Dodds WJ, Jørgensen L, Mustard JF. Experimental hemostasis in normal dogs and dogs with congenital disorders of blood coagulation. *Blood* 1967; 30: 636-68.
- Hudson J, McCaughey WTE. Mural thrombosis and atherogenesis in coronary arteries and aorta: An investigation using antifibrin and antiplatelet sera. *Atherosclerosis* 1974; 19: 543-53.
- Hugues J. Accolement des plaquettes aux structures conjonctives perivasculaires. *Thromb Diath Haemorrh* 1962; 8: 241-55.
- Hugues J, Lapière CM. Nouvelles recherches sur l'accolement des plaquettes aux fibre de collagène. *Thromb Diath Haemorrh* 1964; 11: 327-54.

- Hærem JW. Mural platelet microthrombi and major acute lesions of main epicardial arteries in sudden coronary death. *Atherosclerosis* 1974; 19: 529-41.
- Høygaard A. Studies on nutrition and physiopathology of eskimos. Skrifter utgit av Det Norske Videnskaps Akademi i Oslo. I. Mat-Naturv Klasse, No 9, Oslo, 1940.
- Irion E, Blombäck M. Prostaglandins in platelet aggregation. *Scand J Clin Lab Invest* 1969; 24: 141-4.
- Ivy AC, Nelson D, Bucher G. The standardization of certain factors in the cutaneous »venostasis« bleeding time technique. *J Lab Clin Med* 1941; 26: 1812-22.
- Jaffe EA, Weksler BB. Recovery of endothelial cell prostacyclin production after inhibition by low dose of aspirin. *J Clin Invest* 1979; 63: 532-5.
- Jakubowski JA, Ardlie NG. Evidence for the mechanism by which eicosapentaenoic acid inhibits human platelet aggregation and secretion — implications for the prevention of vascular disease. *Thromb Res* 1979; 16: 205-17.
- Johnson GJ, Leis LA, Rao GHR, White JG: Arachidonate-induced platelet aggregation in the dog *Thromb Res*. 1979a; 14: 147-54.
- Johnson GJ, Schwartz BS, Leis LA: In vivo platelet retention in human bleeding-time wounds. *J Lab Clin Med* 1979 b; 94: 563-73.
- Johnson M, Harrison HE, Raftery AT, Elder JB. Vascular prostacyclin production may be reduced in diabetes in man. *Lancet* 1979c; I: 325-6.
- Johnson RA, Lincoln FH, Thompson JL, Nidy EG, Mizesak SA, Axen U. Synthesis and stereochemistry of prostacyclin and synthesis of 6-keto-prostaglandin $F_{1\alpha}$. *J Am Chem Soc* 1977; 99: 4182-4.
- Johnson RA, Morton DR, Kinner JH, Gorman RR, McGuire JC, Sun FF. The chemical structure of prostaglandin X (prostacyclin). *Prostaglandins* 1976; 12: 915-28.
- Joist JH, Dolezel G, Lloyd JV, Kinlough-Ratbone RL, Mustard JF. Platelet factor-3 availability and the platelet release reaction. *J Lab Clin Med* 1974; 84: 474-82.
- Jørgensen KA. Det hæmolytiske uræmiske syndrom. *Ugeskr Læger* 1978; 140: 1670-2.
- Jørgensen KA, Olesen AS, Dyerberg J, Stoffersen E. Aspirin and bleeding-time: Dependency of age. *Lancet* 1979 b; II: 302.
- Jørgensen KA, Stoffersen E. Dipyridamole and platelet function. *Lancet* 1978; II: 1257-9.
- Jørgensen KA, Stoffersen E. Heparin like activity of albumin. *Thromb Res* 1979c; 16: 569-74.
- Jørgensen KA, Stoffersen E. Antithrombin III and the nephrotic syndrome. *Scand J Haematol* 1979 b; 22: 442-8.
- Jørgensen KA: Stoffersen E, Dyerberg J. Stability of prostacyclin in plasma. *Lancet* 1979 a; I: 1352.

- Jørgensen L, Borchgrevink CF. The platelet plug in normal persons. *Acta Pathol Microbiol Scand* 1963; 57: 40-56.
- Kaneshiro MM, Mielke CH, Kasper CK, Rapaport SI. Bleeding time after aspirin in disorders of intrinsic clotting. *New Engl J Med* 1969; 281: 1039-42.
- Kanfer A, Kleinknecht DD, Broyer M, Josso F. Coagulation studies in 45 cases of nephrotic syndrome without uremia. *Thromb Diath Haemorrh* 1970; 24: 562-71.
- Kaplan BS, Drummond KN. The hemolytic-uremic syndrome is a syndrome. *New Engl J Med* 1978; 298: 964-6.
- Karmazyn M, Horrobin DF, Manku MS, Cuannane SC, Karmali RA, Ally AI, Morgan RD, Nicolaou KC, Barnette NE. Effects of prostacyclin on perfusion pressure, electrical activity, rate and force of contraction in isolated rat and rabbit hearts. *Life Sciences* 1978; 22: 2079-86.
- Kaufmann RH, Veltkamp JJ, van Tilburg NH, van Es LA. Acquired antithrombin III deficiency and thrombosis in the nephrotic syndrome. *Am J Med* 1978; 65: 607-13.
- Kelly RC, Schletter I, Stein SJ. Synthesis of thromboxane B₂. *Tetrahedron Letters* 1976; 37: 3279-82.
- Kelton JG, Hirsh J, Carter CJ, Buchanan MR. Thrombogenic effect of high-dose aspirin in rabbits: Relationship to inhibition of vessel wall synthesis of prostaglandin I₂-like activity. *J Clin Invest* 1978; 62: 892-5.
- Kendall AG, Lohmann RC, Dossetor JB. Nephrotic syndrome. A hypercoagulable state. *Arch Intern Med* 1971; 127: 1021-7.
- Kenimer JG, Goldberg V, Blecher M. The endocrine system: interaction of prostaglandins with adenylyl cyclase-cyclic AMP systems. In: Ramwell PW, ed. *Prostaglandins*, Vol 3, Plenum Press, New York, 1977, pp. 77-108.
- Kinlough-Rathbone RL, Packham MA, Mustard JF. The effect of prostaglandin E₁ on platelet function in vitro and in vivo. *Brit J Haematol* 1970; 19: 559-71.
- Kinlough-Rathbone RL, Reimers HJ, Mustard JF, Packham MA. Sodium arachidonate can induce platelet shape change and aggregation which are independent of the release reaction. *Science* 1976; 192: 1011-2.
- Kitchens CS, Weiss L. Ultrastructural changes of endothelium associated with thrombocytopenia. *Blood* 1975; 46: 567-78.
- Kloeze J. Influence of prostaglandins on platelet adhesiveness and platelet aggregation. In: Bergström S & Samuelson B, Stockholm, eds. *Prostaglandins*, Proceeding of the 2nd Nobel Symposium, Interscience Publishers, New York, 1967, pp. 241-52.
- Kloeze J. Relationship between chemical structure and platelet-aggregation activity of prostaglandins. *Biochim Biophys Acta* 1969; 187: 285-92.

- Korbut R, Moncada S. Prostacyclin (PGI_2) and thromboxane A_2 interaction in vivo. Regulation by aspirin and relationship with antithrombotic therapy. *Thromb Res* 1978; 13: 489-500.
- Kuehl FA, Humes JL, Egan EW, Ham EA, Beveridge GS, van Arman CG. Role of prostaglandin endoperoxide PGG_2 in inflammatory processes. *Nature* 1977; 265: 170-3.
- Kurzrok R, Lieb CC. Biochemical studies of human semen. II. The action of semen on the human uterus. *Proc Soc Exp Biol Med* 1930; 28: 268-72.
- Kutti J, Weinfeld A. Platelet survival and platelet production in acute myocardial infarction. *Acta Med Scand* 1979; 205: 501-4.
- Kwaan HC, Colwell JA, Cruz S, Suwanwela N, Dobbie JG. Increased platelet aggregation in diabetes mellitus. *J Lab Clin Med* 1972; 80: 236-46.
- Käser-Glanzmann R, Jakabova M, George JN, Lüscher EF. Stimulation of calcium uptake in platelet membrane vesicles by adenosine 3',5'-cyclic monophosphate and protein kinase. *Biochim Biophys Acta* 1977; 466: 429-40.
- Lapetina EG, Schmitges CJ, Chandrabose K, Cuatrecasas P. Cyclic adenosine 3',5'-monophosphate and prostacyclin inhibit membrane phospholipase activity in platelets. *Biochem Biophys Res Commun* 1977; 76: 828-35.
- Larrue J, Rigaud M, Daret D, Demond J, Durand J, Bricaud H. Prostacyclin production by cultured smooth muscle cells from atherosclerotic rabbit aorta. *Nature* 1980; 285: 480-2.
- Lee WH, McGriff JC, Householder RW, Sun FF, Wong PY-K. Inhibition of platelet aggregation by 6-keto-prostaglandin E_1 (6-KPGE $_1$), a metabolite of 6-keto-prostaglandin $\text{F}_{1\alpha}$ (6-KPGF $_{1\alpha}$). *Fed Proc* 1979; 38: 419.
- Lewis GP, Priscilla Piper J, Vigo C. The distribution and mobilisation of arachidonic acid in fat cell ghosts and its modification by glucocorticoids. *Brit J Pharmacol* 1979; 66: 99P.
- Lindsay RM, Moorthy AV, Koens F, Linton AL. Platelet function in dialyzed and non-dialyzed patients with chronic renal failure. *Clin Nephrol* 1975; 4: 52-7.
- Lüscher EF, Bettex-Galland M. Thrombostenin, the contractile protein of blood platelets. New facts and problems. *Pathol Biol* 1972; 20 (suppl): 89-101.
- MacIntyre DE, Pearson JD, Gordon JL. Localisation and stimulation of prostacyclin production in vascular cells. *Nature* 1978; 271: 549-51.
- MacMillan DC. Secondary clumping effect in human citrated platelet-rich plasma produced by adenosine diphosphate and adrenaline. *Nature* 1966; 211: 140-4.
- Malik KU. Prostaglandins — modulation of adrenergic nervous system. *Fed Proc* 1978; 37: 203-7.

- Malmsten C, Hamberg M, Svensson J, Samuelsson B. Physiological role of an endoperoxide in human platelets: hemostatic defect due to cyclooxygenase deficiency. *Proc Natl Acad Sci (USA)* 1975; 72: 1446-50.
- Malmsten C, Kindahl H, Samuelsson B, Levy-Toledano S, Tobelem G, Cane J. Thromboxane synthesis and the platelet release reaction in Bernard-Soulier syndrome, thrombasthenia Glanzmann and Hermansky-Pudlak syndrome. *Brit J Haematol* 1977; 35: 511-20.
- Mannucci PM, Pareti FI, Mannucci L. Platelet functions after intravenous administration in man of cyclic-AMP and related drugs. *J Clin Lab Med* 1974; 84: 828-38.
- Marcus AJ. Platelet function. *New Engl J Med* 1969; 280: 1278-84.
- Marcus AJ, Ullman HL, Safier LB. Lipid composition of subcellular particles of human blood platelets. *J Lipid Res* 1969; 10: 108-114.
- Marcus AJ, Weksler BB, Jaffe EA. Enzymatic conversion of prostaglandin endoperoxide H_2 and arachidonic acid to prostacyclin by cultured human endothelial cells. *J Biol Chem* 1978; 253: 7138-41.
- Markelonis G, Garbus J. Alterations of intracellular oxidative metabolism as stimuli evoking prostaglandin biosynthesis: A review of prostaglandins in cell injury and a hypothesis. *Prostaglandins* 1975; 10: 1087-1106.
- Marquis NR, Vigdahl RL, Tavormina PA. Platelet aggregation. I. Regulation by cyclic AMP and prostaglandin E_1 . *Biochem Biophys Res Commun* 1969; 36: 965-72.
- Martin JF, Suggett AJ, Leach E, Moncada S. The effect of prostacyclin on platelet aggregation and disaggregation in vivo. *Thromb Res* 1980; 18: 749-51.
- Masotti G, Galanti G, Poggesi L, Abbate R, Serneri GGN. Differential inhibition of prostacyclin production and platelet aggregation by aspirin. *Lancet* 1979 a; II: 1213-6.
- Masotti G, Poggesi L, Galanti G, Serneri GGN. Stimulation of prostacyclin by dipyridamole. *Lancet* 1979 b; I: 1412.
- Massini P, Käser-Glanzmann R, Lüscher EF. Movement of calcium ions and their role in the activation of platelets. *Thrombos Haemostas* 1978; 40: 212-8.
- Massini P, Lüscher EF. On the significance of the influx of calcium into stimulated human blood platelets. *Biochim Biophys Acta* 1976; 436: 652-63.
- Mathé AA. Prostaglandins and the lung. In: Ramwell PW, ed. *Prostaglandins*, Vol 3, Plenum Press, New York, 1977, pp. 169-224.
- Mayne EE, Bridges JM, Weaver JA. Platelet adhesiveness, plasma fibrinogen and factor VIII levels in diabetes mellitus. *Diabetologia* 1970; 6: 436-440.

- McGriff JC, Terragno NA, Strand JC, Lee JB, Lonigro AJ, Ng KK. Selective passage of prostaglandins across the lung. *Nature* 1969; 223: 742-5.
- McMillan RM, MacIntyre DE, Booth A, Gordon JL. Malonaldehyde formation in intact platelets is catalysed by thromboxane synthetase. *Biochem J* 1978; 176: 595-8.
- Meyers KM, Seachord CL, Holmsen H, Smith JB, Prieur DJ. A dominant role of thromboxane formation in secondary aggregation of platelets. *Nature* 1979; 282: 331-3.
- Miller OV, Aiken JW, Shebuski RJ, Gorman RR. 6-keto-prostaglandin E_1 is not equipotent to prostacyclin (PGI_2) as an antiaggregatory agent. *Prostaglandins* 1980; 20: 391-400.
- Mills DCB, MacFarlane DE. Stimulation of human platelet adenylate cyclase by prostaglandin D_2 . *Thromb Res* 1974; 5: 401-12.
- Mills DCB, MacFarlane DE. Prostaglandins and platelet adenylate cyclase. In: Silver MJ, Smith JB, Kocsis JJ eds. *Prostaglandins in hematology*. Spectrum Publications Inc., New York, 1977, pp. 219-34.
- Mills DCB, Robb IA, Roberts GCK. The release of nucleotides, 5-hydroxytryptamine and enzymes from human platelets during aggregation. *J Physiol (London)* 1968; 195: 715-29.
- Mills DCB, Smith JB. The influence on platelet aggregation of drugs that effect the accumulation of adenosine 3':5'-cyclic monophosphate in platelets. *Biochem J* 1971; 121: 185-96.
- Mills DCB, Smith JB. The control of platelet responsiveness by agents that influence cyclic AMP metabolism. *Ann NY Acad Sci* 1972; 201: 391-9.
- Minkes MS, Joist JH, Needleman P. Arachidonic acid-induced platelet aggregation independent of ADP-release in a patient with a bleeding disorder due to platelet storage pool disease. *Thromb Res* 1979; 15: 169-179.
- Mitchell JRA, Sharp AA. Platelet clumping in vitro. *Brit J Haematol* 1964; 10: 78-93.
- Miyamoto T, Ogino N, Yamamoto S, Hayaishi C. Purification of prostaglandin endoperoxide synthetase from bovine vesicular gland microsomes. *J Biol Chem* 1976; 251: 2629-36.
- Moncada S, Bunting S, Mullane K, Thorogod P, Vane JR, Ruz A, Needleman P. Imidazole: A selective inhibitor of thromboxane synthetase. *Prostaglandins* 1977 c; 13: 611-8.
- Moncada S, Ferreira SH, Vane JR. Prostaglandins, aspirin-like drugs and oedema of inflammation. *Nature* 1973; 246: 217-9.
- Moncada S, Gryglewski R, Bunting S, Vane JR. An enzyme isolated from arteries transforms prostaglandin endoperoxides to an unstable substance that inhibits platelet aggregation. *Nature* 1976a; 263: 663-5.

- Moncada S, Gryglewski RJ, Bunting S, Vane JR. A lipid peroxide inhibits the enzyme in blood vessel microsomes that generates from prostaglandin endoperoxides the substance (prostaglandin X) which prevents platelet aggregation. *Prostaglandins* 1976 c; 12: 715-37.
- Moncada S, Herman AG, Higgs EA, Vane JR. Differential formation of prostacyclin (PGX or PGI₂) by layers of arterial wall. An explanation for the antithrombotic properties of vascular endothelium. *Thromb Res* 1977 b; 11: 323-44.
- Moncada S, Higgs EA, Vane JR. Human arterial and venous tissue generate prostacyclin (prostaglandin X), a potent inhibitor of platelet aggregation. *Lancet* 1977 a; 1: 18-20.
- Moncada S, Korbut R. Dipyridamole and other phosphodiesterase inhibitors act as antithrombotic agents by potentiating endogenous prostacyclin. *Lancet* 1878 b; 1: 1286-9.
- Moncada S, Korbut R, Bunting S, Vane JR. Prostacyclin is a circulating hormone. *Nature* 1978 a; 273: 767-8.
- Moncada S, Mulane KM, Vane JR. Prostacyclin-release by bradykinin in vivo. *Brit J Pharmacol* 1979; 66: 96P-97P.
- Moncada S, Needleman P, Bunting S, Vane JR. Prostaglandin endoperoxide and thromboxane generating systems and their selective inhibition. *Prostaglandins* 1976 b; 12: 323-35.
- Moore S, Friedman RJ, Singal DP, Gaudie J, Blajchman MA, Roberts RS. Inhibition of injury induced thromboatherosclerotic lesions by anti-platelet serum in rabbits. *Thrombos Haemostas* 1976; 35: 70-81.
- Moskowitz J, Harwood JP, Reid WD, Krishna G. The interaction of norepinephrine and prostaglandin E₁ on the adenylyl cyclase system of human and rabbits blood platelets. *Biochim Biophys Acta* 1971; 230: 279-85.
- Mustard JF, Packham MA. Thromboembolism: a manifestation of the response of blood to injury. *Circulation* 1970; 42: 1-21.
- Mustard JF, Packham MA. Platelets and diabetes mellitus. *New Engl J Med* 1977; 297: 1345-7.
- Myatt L, Elder MG. Inhibition of platelet aggregation by a placental substance with prostacyclin-like activity. *Nature* 1977; 268: 159-60.
- Nachman RL, Weksler B, Ferris B. Characterization of human platelet vascular permeability-enhancing activity. *J Clin Invest* 1972; 51: 549-56.
- Needleman P. Prostacyclin in blood vessel-platelet interactions: perspectives and questions. *Nature* 1979; 279: 14-15.
- Needleman P, Bryan B, Wyche A, Bronson S, Eakins K, Ferrendelli J, Minkes M. Thromboxane synthetase inhibitors as pharmacological tools: Differential biochemical and biological effects on platelet suspensions. *Prostaglandins* 1977 b; 14: 897-907.

- Needleman P, Minkes M, Raz A. Thromboxanes: Selective biosynthesis and distinct biological properties. *Science* 1976 b; 193: 163-5.
- Needleman P, Moncada S, Bunting S, Vane JR, Hamberg M, Samuelsson B. Identification of an enzyme in platelet microsomes which generates thromboxane A₂ from prostaglandin endoperoxides. *Nature* 1976 a; 261: 558-60.
- Needleman P, Raz A, Ferrendelli JA, Minkes M. Application of imidazole as a selective inhibitor of thromboxane synthetase in human platelets. *Proc Natl Acad Sci (USA)* 1977a; 74: 1716-20.
- Needleman P, Raz A, Minkes MS, Ferrendelli JA, Sprecher H. Triene prostaglandins: Prostacyclin and thromboxane biosynthesis and unique biological properties. *Proc Natl Acad Sci (USA)* 1979 b; 76: 944-8.
- Needleman P, Wyche A, Raz A. Platelet and blood vessel arachidonate metabolism and interactions. *J Clin Invest* 1979 a; 345-9.
- Nelson AN, Jackson RW. Total synthesis of thromboxane B₂. *Tetrahedron Letters* 1976; 37: 3275-8.
- Nelson WR, Taylor GA. In vitro inhibition of endotoxin induced platelet aggregation with hydrocortisone sodium succinate (Solu-Cortef®). *Scand J Haematol* 1975; 15: 35-44.
- Nenci GG, Berretini M, Agnelli G, Parise P, Buoneristiani U, Ballatori E. Effect of peritoneal dialysis, haemodialysis and kidney transplantation on blood platelet function. *Nephron* 1979; 23: 287-92.
- Nicolaou KC, Magolda RL, Smith JB, Aharony D, Smith EF, Lefer AM. Synthesis and biological properties of pinane-thromboxane A₂, a selective inhibitor of coronary artery constriction, platelet aggregation, and thromboxane formation. *Proc Natl Acad Sci (USA)* 1979; 76: 2566-70.
- Nishizawa EE, Miller WL, Gorman RR, Bundy GL, Svensson J, Hamberg M. Prostaglandin D₂ as a potential anti-thrombotic agent. *Prostaglandins* 1975; 9: 109-19.
- Nordøy A. Albumin-bound fatty acids, platelets and endothelial cells in thrombogenesis. *Haemostasis* 1979; 8: 193-202.
- Nordøy A, Svensson B, Hoak JC. The inhibitory effect of human endothelial cell monolayers on platelet reactions and its inhibition by aspirin. *Thromb Res* 1978; 12: 597-608.
- Nugteren DH, Hazelhof E. Isolation and properties of intermediates in prostaglandin biosynthesis. *Biochim Biophys Acta* 1973; 216: 448-61.
- O'Brien JR. Some effects of adrenaline and anti-adrenaline compounds on platelets in vitro and in vivo. *Nature* 1963; 200: 763-4.
- O'Brien JR. Effects of adenosine diphosphate and adrenaline on mean platelet shape. *Nature* 1965; 207: 306-7.
- O'Brien JR. Effect of salicylates on human platelets. *Lancet* 1968; i: 779-83.

- O'Brien JR, Finch W, Clark E. A comparison of an effect of different anti-inflammatory drugs on human platelets. *J Clin Path* 1970; 23: 522-25.
- Oelz O, Oelz R, Knapp HR, Sweetman BJ, Oates JA. Formation of prostaglandin D₂ by human platelets. *Clinical Research* 1976a; 24: 17A.
- Oelz O, Oelz R, Knapp HR, Sweetman BJ, Oates JA. Biosynthesis of prostaglandin D₂. 1. Formation of prostaglandin D₂ by human platelets. *Prostaglandins* 1977; 13: 225-34.
- O'Grady J, Moncada S. Aspirin: A paradoxical effect on bleeding time. *Lancet* 1978; II: 780.
- Okuma M, Takayama H, Uchino H. Generation of prostacyclin-like substance and lipid peroxidation in vitamin E-deficient rats. *Prostaglandins* 1980; 19: 527-36.
- van der Ouderaa FJ, Buytenhek M, Nugteren DH, van Dorp DA. Purification and characterisation of prostaglandin endoperoxide synthetase from sheep vesicular glands. *Biochim Biophys Acta* 1977; 487: 315-31.
- Pace-Asciak C. A new prostaglandin metabolite of arachidonic acid. Formation of 6-keto-PGF_{1α} by rat stomach. *Experientia* 1976 a; 32: 291-2.
- Pace-Asciak C. Isolation, structure and biosynthesis of 6-ketoprostaglandin F_{1α} in the rat stomach. *J Am Chem Soc* 1976 b, 98: 2348-9.
- Pace-Asciak C, Carrara MC, Domazet Z. Identification of the major urinary metabolites of 6-ketoprostaglandin F_{1α} in the rat. *Biophys Res Commun* 1977; 78: 115-21.
- Pace-Asciak C, Nashat M. Mechanistic studies on the biosynthesis of 6-ketoprostaglandin F_{1α}. *Biochim Biophys Acta* 1977; 487: 495-507.
- Pace-Asciak C, Wolfe LS. A novel prostaglandin derivative formed from arachidonic acid by rat stomach homogenates. *Biochemistry* 1971; 10: 3657-64.
- Packham MA, Kinlough-Ratbone RL, Reimers HJ, Scott S, Mustard JF. Mechanisms of platelet aggregation independent of adenosine diphosphate. In: Silver MJ, Smith JB, Kocsis JJ, eds. *Prostaglandins in Hematology*, Spectrum, New York 1977, pp. 247-76.
- Packham MA, Mustard JF. Clinical pharmacology of platelets. *Blood* 1977; 50: 555-73.
- Packham MA, Nishizawa EE, Mustard JF. Response of platelets to tissue injury. *Biochem Pharmacol* 1968; (suppl): 171-84.
- Parbtani A, Cameron JS. Platelet and plasma serotonin concentrations in glomerulonephritis I. *Thromb Res* 1979; 15: 109-25.
- Pareti FI, Mannucci PM, D'Angelo A, Smith JB, Sautebin L, Galli G. Congenital deficiency of thromboxane and prostacyclin. *Lancet* 1980; I: 898-901.

- Pavlovsky M, Yipintsoi T, Didisheim P. Factors affecting arterial thrombogenesis. *Clin Res* 1971; 19: 332.
- Pedersen AK. Dipyridamole and platelet aggregation. *Lancet* 1978; II: 270.
- Pickett WC, Jesse RL, Cohen P. Initiation of phospholipase A₂ activity in human platelets by the calcium ion ionophore A 23187. *Biochim Biophys. Acta* 1977; 486: 209-13.
- Pierce CH, Oshiro G, Nicherson M. Effects of methylprednisolone sodium succinate (MP) on platelet aggregation. *Circulation* 1974; 49: (suppl I), III-289.
- Pinninger JL, Prunty FTG. Some observations on the blood-clotting mechanism, role of fibrinogen and platelets, with reference to a case of congenital afibrinogenemia. *Brit J Exp Pathol* 1946; 27: 200-10.
- Piper PJ, Vane JR. Release of additional factors in anaphylaxis and its antagonism by anti-inflammatory drugs. *Nature* 1969; 223: 29-35.
- Piper P, Vane J. The release of prostaglandins from lung and other tissues. *Ann NY Acad Sci* 1971; 180: 363-85.
- Piper PJ, Vane JR, Wyllie JH. Inactivation of prostaglandins by the lungs. *Nature* 1970; 225: 600-4.
- Porta M, Hilgard P, Kohner EM. Platelet shape change abnormalities in diabetic retinopathy. *Diabetologia* 1980; 18: 217-21.
- Powell EDU, Field RA. Diabetic retinopathy and rheumatoid arthritis. *Lancet* 1964; II: 17-8.
- Preston FE, Ward JD, Marcola B, Porter NR, Timperly WR, O'Malley BC. Elevated β -thromboglobulin levels and circulating platelet aggregates in diabetic microangiopathy. *Lancet* 1978; I: 238-9.
- Puré E, Needleman P. Effect of endothelial damage on prostaglandin synthesis by isolated perfused rabbit mesenteric vasculature. *J Cardiovasc Pharmacol* 1979; 1: 299-309.
- Quick AJ. Acetylsalicylic acid as a diagnostic aid in hemostasis. *Am J Med Sci* 1967; 254: 392-7.
- Quilley CP, McGiff JC, Lee WH, Sun FF, Wong PYK. 6-keto-PGE₁: A possible metabolite of prostacyclin having platelet antiaggregatory effects. *Hypertension* 1980; 2: 524-8.
- Quilley CP, Wong PYK, McGiff JC. Hypotensive and renovascular actions of 6-keto-prostaglandin E₁, a metabolite of prostacyclin. *Europ J Pharmacol* 1979; 57: 273-6.
- Rak K, Boda Z. Haemostatic balance in congenital deficiency of platelet cyclo-oxygenase. *Lancet* 1980; II: 44.
- Rasmussen H. Cell communication, calcium ion, and cyclic adenosine monophosphate. *Science* 1970; 170: 404-12.
- Remuzzi G, Cavenaghi AE, Mecca G, Donati MB, deGaetano G. Prostacyclin-like activity and bleeding in renal failure. *Lancet* 1977; II: 1195-7.

- Remuzzi G, Livio M, Cavenaghi AE, Marchesi D, Mecca G, Donati MB, deGaetano G. Unbalanced prostaglandin synthesis and plasma factors in uraemic bleeding. A hypothesis. *Thromb Res* 1978 a; 13: 5311-6.
- Remuzzi G, Marchesi D, Cavenaghi AE, Livio M, Donati MB, deGaetano G, Mecca G. Bleeding in renal failure: A possible role of vascular prostacyclin (PGI₂). *Clin Nephrol* 1979 a; 12: 127-31.
- Remuzzi G, Marchesi D, Livio M, Cavenaghi AE, Mecca G, Donati MB, deGaetano G. Altered platelet and vascular prostaglandin-generation in patients with renal failure and prolonged bleeding times. *Thromb Res* 1978 b; 13: 1007-15.
- Remuzzi G, Marchesi D, Misiani R, Mecca G, deGaetano G, Donati MB. Familial deficiency of a plasma factor stimulating vascular prostacyclin activity. *Thromb Res* 1979 b; 16: 517-25.
- Remuzzi G, Mecca G, Livio M, deGaetano G, Donati MB, Pearson JD, Gordon JL: Prostacyclin generation by cultured endothelial cells in haemolytic uraemic syndrome. *Lancet* 1980; I: 656-7.
- Remuzzi G, Mecca G, Marchesi D, Livio M, deGaetano G, Donati MB, Silver MJ. Platelet hyperaggregability and the nephrotic syndrome. *Thromb Res* 1979 d; 16: 345-54.
- Remuzzi G, Misiani R, Marchesi D, Livio M, Mecca G, deGaetano G, Donati MB. Haemolytic-uremic syndrome: Deficiency of plasma factor(s) regulating prostacyclin activity? *Lancet* 1978 c; II: 871-2.
- Remuzzi G, Misiani R, Marchesi D, Livio M, Mecca G, deGaetano G, Donati MB. Treatment of the hemolytic uremic syndrome with plasma. *Clin Nephrol* 1979 c; 12: 279-84.
- Riella C, George CRP, Hickman RO, Striker GB, Slichter SJ, Harker L, Quadracci LJ. Renal micro-angiopathy of the hemolytic-uremic syndrome in childhood. *Nephron* 1976; 17: 188-203.
- Rittenhouse-Simmons S. Production of diglyceride from phosphatidylinositol in activated human platelets. *J Clin Invest* 1979; 63: 580-7.
- Rittenhouse-Simmons S, Deykin D. The activation by Ca²⁺ of platelet phospholipase A₂. Effects of dibutyrylcyclic adenosine monophosphate and 8-(N,N-diethylamino)-octyl-3,4,5-trimethoxybenzoate. *Biochim Biophys Acta* 1978; 543: 409-22.
- Rittenhouse-Simmons S, Russel FA, Deykin D. Mobilization of arachidonic acid in human platelets, kinetics and Ca⁺⁺ dependency. *Biochim Biophys Acta* 1977; 488: 370-80.
- Robert A. Prostaglandins and the digestive system. In: Ramwell PW, ed. *Prostaglandins*, Vol 3, Plenum Press, New York, 1977; pp. 225-66.

- Rome LH, Lands WEM. Aspirin as a quantitative acetylation reagent for the fatty acid oxygenase that forms prostaglandins. *Prostaglandins* 1976; 11: 23-30.
- Ross R, Vogel A. The platelet-derived growth factor. *Cell* 1978; 14: 203-10.
- Ross R, Glomset J, Kariya B, Harker LA: A platelet-dependent serum factor that stimulates the proliferation of arterial smooth muscle cells in vitro. *Proc. Natl. Acad. Sci (USA)* 1974; 71: 1207-10.
- Rossi EC, Levin NW. Inhibition of ADP-induced platelet aggregation by furosemide. *J Lab Clin Med* 1973; 81: 140-7.
- Roth GJ, Majerus PW. The mechanism of the effect of aspirin on human platelets. 1. Acetylation of a particulate fraction protein. *J Clin Invest* 1975; 56: 624-32.
- Roth GJ, Stanford N, Majerus PW. Acetylation of prostaglandin synthetase by aspirin. *Proc Natl Acad Sci (USA)* 1975; 72: 3073-6.
- Rowland M, Riegelman S, Harris PA, Sholkoff SD. Absorption kinetics of aspirin in man following oral administration of an aqueous solution. *J Pharm Sci* 1972; 61: 379-85.
- Russel FA, Deykin D. The effect of thrombin on the uptake and transformation of arachidonic acid by human platelets. *Am J Haematol* 1976; 1: 59-70.
- Sagel J, Colwell JA, Crook L, Laimins M. Increased platelet aggregation in early diabetes mellitus. *Ann Int Med* 1975; 82: 733-8.
- Salky N, Dugdale M. Platelet abnormalities in ischemic heart disease. *Am J Cardiol* 1973; 32: 612-7.
- Salmon JA, Smith DR, Flower RJ, Moncada S, Vane JR. Further studies on the enzymatic conversion of prostaglandin endoperoxide into prostacyclin by porcine aorta microsomes. *Biochim Biophys Acta* 1978; 523: 250-62.
- Salzman EW. Cyclic AMP and platelet function. *N Engl J Med* 1972; 286: 358-63.
- Salzman EW. Prostaglandins, cyclic AMP and platelet function. *Thromb Diath Haemorrh* 1974; 60: (suppl): 311-9.
- Salzman EW, MacIntyre DE, Steer ML, Gordon JL. Effect on platelet activity of inhibition of adenylate cyclase. *Thromb Res* 1978; 13: 1089-1101.
- Salzman EW, Neri LL. Cyclic 3',5'-adenosine monophosphate in human blood platelets. *Nature* 1969; 224: 609-10.
- Samuelsson B. On the incorporation of oxygen in the conversion of 8,11,14 eicosatrienoic acid to prostaglandin E₁. *J Am Chem Soc* 1965; 87: 3011-3.
- Samuelsson B. Biosynthesis of prostaglandins. *Fed Proc* 1972; 31: 1442-50.
- Samuelsson B. The role of prostaglandin endoperoxides and thromboxanes in human platelets. In: Silver MJ, Smith JB,

- Kocsis JJ, eds. Prostaglandins in hematology. Spectrum Publications Inc, New York, 1977; pp 1-10.
- Samuelsson B. Prostaglandins and thromboxanes. In: Greep RD, ed. Recent Progress in Hormone Research. Academic Press, New York, 1978; pp. 239-58.
- Sanders TAB, Naismith DJ, Haines AP, Vickers M. Cod-liver oil, platelet fatty acids, and bleeding time. *Lancet* 1980; I: 1189.
- Sanders WJ, Rosenberg A, Hawiger J. Human platelet function under influence of methylprednisolone: Inhibition of aggregation, serotonin release, and clotpromoting phospholipid irrespective of membrane receptors involved. *Clinical Research* 1976; 24: 48A.
- Schneider WP, Morge RA. A synthesis of crystalline thromboxane B₂ from a derivative of prostaglandin F_{2α}. *Tetrahedron* 1976; 37: 3283-6.
- Schoene NW, Iacano JM. Stimulation of platelet phospholipase A₂ activity by aggregating agents. *Fed Proc* 1975; 34: 257.
- Schwartz HC, King KC, Schwartz AL, Edmunds D, Schwartz R. Effects of pregnancy on hemoglobin A_{1c} in normal, gestational diabetic, and diabetic women. *Diabetes* 1976; 25: 1118-22.
- Sekhar NC. Effect of eight prostaglandins on platelet aggregation. *J Med Chem* 1970; 13: 39-44.
- Shio H, Plasse AM, Ramwell PW. Platelet swelling and prostaglandins. *Microvasc Res* 1970; 2: 294-301.
- Shio H, Ramwell P. Effect of prostaglandin E₂ and aspirin on the secondary aggregation of human platelets. *Nature New Biol* 1972; 236: 45-6.
- Siess W, Roth P, Scherer S, Kurzmann I, Böhlig B, Weber PC. Platelet-membrane fatty acids, platelet aggregation, and thromboxane formation during a mackerel diet. *Lancet* 1980; I: 441-4.
- Silberbauer K, Schernthaner G, Sinzinger H, Piza-Katzer H, Winther M. Decreased vascular prostacyclin in juvenile-onset diabetes. *New Engl J Med* 1979c; 300: 366-7.
- Silberbauer K, Sinzinger H, Winter M. Prostacyclin production by vascular smooth-muscle cells. *Lancet* 1978 a; I: 1356-7.
- Silberbauer K, Sinzinger H, Winter M. Increased prostacyclin (PGI₂) availability of vascular tissue in experimentally-induced acute uremic rats. *Artery* 1978 b, 4: 554-63.
- Silberbauer K, Sinzinger H, Winter M. Prostacyclin activity in rat kidney stimulated by angiotensin II. *Br J Exp Path* 1979 a; 60: 38-44.
- Silberbauer K, Sinzinger H, Winter M. Prostacyclin (PGI₂) activity in rat kidney — stimulated by angiotensin II. *Exp Mol Path* 1979 b; 30: 242-54.

- Silver MJ, Hoch W, Kocsis JJ, Ingberman CM, Smith JB. Arachidonic acid causes sudden death in rabbits. *Science* 1974; 183: 1085-7.
- Silver MJ, Smith JB, Ingberman C, Kocsis JJ. Arachidonic acid-induced human platelet aggregation and prostaglandin formation. *Prostaglandins* 1973; 4: 863-75.
- Sinzinger H, Klein K, Kaliman J, Silberbauer K, Feigl W. Enhanced prostacyclin formation in veins of women under chronic treatment with oral contraceptive drugs. *Pharmacol Res Commun* 1980; 12: 515-21.
- Smith JB, Ingberman C, Kocsis JJ, Silver MJ: Formation of an intermediate in prostaglandin biosynthesis and its association with the platelet release reaction. *J Clin Invest* 1974 a; 53: 1468-73.
- Smith JB, Ingberman CM, Silver MJ. Prostaglandins and precursors in platelet function. In: *Biochemistry and Pharmacology of Platelets*. Ciba Foundation Symposium 35 (new series). Elsevier/Excerpta Medica/North-Holland, Amsterdam, 1975; pp. 207-24.
- Smith JB, Ingberman CM, Silver MJ. Malondialdehyde formation as an indicator of prostaglandin production by human platelets. *J Lab Clin Med* 1976; 88: 167-72.
- Smith JB, Ingberman CM, Silver MJ. Effects of arachidonic acid and some of its metabolites on platelets. In: Silver MJ, Smith JB, Kocsis JJ, eds. *Prostaglandins in hematology*. Spectrum Publications Inc., New York, 1977, pp. 277-92.
- Smith JB, MacFarlane DE. Platelets. In: Ramwell PW, ed. *Prostaglandins Vol. 2*, Plenum Press, New York, 1974; pp. 293-43.
- Smith JB, Silver MJ, Ingberman CM, Kocsis JJ. Prostaglandin D₂ inhibits the aggregation of human platelets. *Thromb Res* 1974b; 5: 291-4.
- Smith DR, Weatherly BC, Salmon JA, Ubatuba FB, Gryglewski RJ, Moncada S. Preparation and biochemical properties of PGH₃. *Prostaglandins* 1979; 18: 423-38.
- Smith JB, Willis AL. Formation and release of prostaglandins by platelets in response to thrombin. *Br J Pharmacol* 1970; 40: 545P.
- Smith JB, Willis AL. Aspirin selectively inhibits prostaglandin production in human platelets. *Nature (New Biology)* 1971; 231: 236-7.
- Spaet TH, Erichson RB. The vascular wall in the pathogenesis of thrombosis. *Thromb Diath Haemorrh* 1966; 21: (suppl), 67-86.
- Steele P, Rainwater J, Vogel R. Abnormal platelet survival time in men with myocardial infarction and normal coronary arteriogram. *Am J Cardiol* 1978; 41: 60-2.
- Steer ML, MacIntyre DE, Levine L, Salzman EW. Is prostacyclin a physiologically important circulating anti-platelet agent? *Nature* 1980; 283: 194-5.

- Stormorken H. The release reaction of secretion: a general basic phenomenon related to phagocytosis/pinocytosis. *Scand J Haematol* 1969; 9 (suppl), 3-24.
- Sun FF. Biosynthesis of thromboxane in human platelets, I. Characterization and assay of thromboxane synthetase. *Biochem Biophys Res Comm* 1977; 74: 1432-40.
- Sun FF, Taylor BM. Metabolism of prostacyclin in rat. *Biochemistry* 1978; 17: 4096-4101.
- Svensson J. Biosynthesis and biological properties of prostaglandin endoperoxides and thromboxane A₂. *Acta Biol Med Germ* 1978; 37: 731-40.
- Svensson J, Hamberg M, Samuelsson B. Prostaglandin endoperoxides IX. Characterization of rabbit aorta contracting substance (RCS) from quinea pig lung and human platelets. *Acta Physiol Scand* 1975; 94: 222-8.
- Szczeklik A, Gryglewski RJ, Grodzinska L, Musial J, Serwonska M, Marcinkiewicz E. Platelet aggregability, thromboxane A₂ and malonaldehyde formation following administration of aspirin to man. *Thromb Res* 1979 b; 15: 405-13.
- Szczeklik A, Gryglewski RJ, Nizankowski R, Musial J, Pieton R, Mruk J. Circulatory and anti-platelet effects of intravenous prostacyclin in healthy men. *Pharmacol Res Comm* 1978 a; 10: 545-56.
- Szczeklik A, Gryglewski RJ, Nizankowska E, Nizankowski R, Musial J. Pulmonary and anti-platelet effects of intravenous and inhaled prostacyclin in man. *Prostaglandins* 1978 b; 16: 651-81.
- Szczeklik A, Nizankowski R, Skawinski S, Szczeklik J, Glusko P, Gryglewski RJ. Successful therapy of advanced arteriosclerosis obliterans with prostacyclin. *Lancet* 1979 a; I: 1111-4.
- Tansik RL, Namm DH, White HL. Synthesis of prostaglandin 6-keto-PGF_{1α} by cultured aortic smooth muscle cells and stimulation of its formation in a coupled system with platelet lysates. *Prostaglandins* 1978; 15: 399-408.
- Tateson JE, Moncada S, Vane JR. Effects of prostacyclin (PGX) on cyclic AMP concentrations in human platelets. *Prostaglandins* 1977; 13: 389-97.
- Thaler F, Balzar E, Kopsa H, Pinggera WF. Acquired antithrombin III deficiency in patients with glomerular proteinuria. *Haemostasis* 1978; 7: 257-72.
- Thomsen C, Forbes CS, Prentice CRM, Kennedy AC. Changes in blood coagulation and fibrinolysis in the nephrotic syndrome. *QJ Med* 1974; 43: 399-407.
- Thong KL, Mant MJ, Grace MG. Lack of effect of prednisone administration on bleeding time and platelet function of normal subjects. *Brit J Haemat* 1978; 38: 373-80.
- Trivelli LA, Ranney HM, Lai H. Hemoglobin components in patients with diabetes mellitus. *New Engl J Méd* 1971; 284: 353-7.

- Ubatuba FB, Moncada S, Vane JR. The effect of prostacyclin (PGI_2) on platelet behaviour. Thrombus formation in vivo and bleeding time. *Thromb Haemostas* 1979; 41: 425-35.
- Udén A, Nilsson IM. Correlation between the bleeding time and the platelet aggregating activity of collagen in scoliosis. *Thromb Res* 1979; 16: 93-5.
- Vaage J. Pharmacological doses of corticosteroids and intravascular platelet aggregation in cats. *Microvascular Research* 1978; 15: 113.
- Valdorf-Hansen F. Nogle undersøgelser over trombocyt- og koagulationsforhold hos diabetikere (thesis), Copenhagen, 1967.
- Walsh PN. Platelet coagulant activities and hemostasis: a hypothesis. *Blood* 1974; 43: 597-605.
- Vane JR. Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nature (New Biology)* 1971; 231: 232-5.
- Vane JR. The mode of action of aspirin and similar compounds. *J Allergy Clin Immunol* 1976; 58: 691-712.
- Vane JR. The mode of action of aspirin-like drugs. *Agents and Actions* 1978; 8: 430-1.
- Wardle EN. The role of platelets in glomerulonephritis and transplantation. *J Roy Phys Lond* 1972; 7: 5-18.
- Wasserman MA. Prostaglandins: Birth of a new therapeutic era. *American Pharmacy* 1979; 19: 15-19.
- Weily HS, Steele PP, Davies H, Pappas G, Genton E. Platelet survival in patients with substitute heart valves. *New Engl J Med* 1974; 290: 534-6.
- Weiss JW, Aledort LM, Kochwa S. The effect of salicylates on the hemostatic properties of platelets in man. *J Clin Invest* 1968; 47: 2169-80.
- Weiss SJ, Turk J, Needleman P. A mechanism for the hydroperoxide-mediated inactivation of prostacyclin synthetase. *Blood* 1979; 53: 1191-6.
- Weksler BB, Ley CW, Jaffe EA. Stimulation of endothelial cell prostacyclin (PGI_2) production by thrombin, trypsin and the ionophore A 23187. *J Clin Invest* 1978; 62: 923-30.
- Weksler BB, Marcus AJ, Jaffe EA. Synthesis of prostaglandin I_2 (prostacyclin) by cultured human and bovine endothelial cells. *Proc Natl Acad Sci (USA)* 1977; 74: 3922-6.
- Vermynen J, Chamone DAF, Verstraete M. Stimulation of prostacyclin release from vessel wall by Bay g 6575, an anti-thrombotic compound. *Lancet* 1979; I: 518-20.
- Wester J, van der Veen J, Sixma JJ. Morphology of the haemostatic plug. In: Mills DCB, Parodi FI, eds. *Platelets and Thrombosis*. Academic Press, New York, 1977; pp: 97-109.
- White JG. Fine structural alterations induced in platelets by adenosine diphosphate. *Blood* 1968; 31: 604-21.

- White JG, Rao GHR, Gerrard JM. Effects of the ionophore A23187 on blood platelets. I. Influence on aggregation and secretion. *Am J Path* 1974; 77: 135-50.
- Whittaker N. A synthesis of prostacyclin sodium salt. *Tetrahedron Letters* 1977; 32: 2805-8.
- Whittaker MO, Wyche A, Fitzpatrick F, Sprecher H, Needleman P. Triene prostaglandins: Prostaglandin D₃ and icosapentaenoic acid as potential antithrombotic substances. *Proc Natl Acad Sci (USA)* 1979; 76: 5919-23.
- Whittle BJR, Moncada S, Vane JR. Comparison of the effects of prostacyclin (PGI₂), prostaglandin E₁ and D₂ on platelet aggregation in different species. *Prostaglandins* 1978; 16: 373-88.
- Vigdahl RL, Marquis NR, Tavormina PA. Platelet aggregation. II. Adenyl cyclase, prostaglandin E₁ and calcium. *Biochim Biophys Res Commun* 1969; 37: 409-15.
- Villa S, Callioni A, de Gaetano G. Normal prostacyclin-like activity in vascular tissues from thrombocytopenic rats. *Thromb Res* 1977 b; 11: 701-4.
- Villa S, Mysliwiec M, de Gaetano G. Prostacyclin and atherosclerosis in rats. *Lancet* 1977 a; I: 1216-7.
- Willems C, van Aken WG. Production of prostacyclin by vascular endothelial cells. *Haemostasis* 1979; 8: 266-73.
- Willis AL. Isolation of a chemical trigger for thrombosis. *Prostaglandins* 1974 a; 5: 1-25.
- Willis AL. An enzymatic mechanism for the antithrombotic and antihemostatic actions of aspirin. *Science* 1974b; 183: 325-7.
- Willis AL, Vane FM, Kuhn DC, Scott GG, Petrin M. An endoperoxide aggregator (LASS) formed in platelets in response to thrombotic stimuli. Purification, identification and unique biological significance. *Prostaglandins* 1974; 8: 453-507.
- Wilson RB. Lipid peroxidation and atherosclerosis. *Crit Rev Food Sci Nutr* 1976; 8: 325-38.
- Wlodawer P, Samuelsson B. On the organization and mechanism of prostaglandin synthetase. *J Biol Chem* 1973; 248: 5673-8.
- Wong PY-K, McGriff JC, Cagen L, Malik KU, Sun FF. Metabolism of prostacyclin in the rabbit kidney. *J Biol Chem* 1979; 254: 12-14.
- Wong PY-K, Sun FF, McGriff JC. Metabolism of prostacyclin in blood vessels. *J Biol Chem* 1978; 253: 5555-7.
- Wright JH, Minot GR. The viscous metamorphosis of blood platelets. *J Exp Med* 1917; 26: 395-409.
- Yoshida N, Aoki N. Release of arachidonic acid from human platelets. A key role for the potentiation of platelet aggregability in normal subjects as well as those with nephrotic syndrome. *Blood* 1978; 52: 969-77.

- Zmuda A, Dembinska-Kiec A, Chytkowski A, Gryglewski RJ. Experimental atherosclerosis in rabbits: Platelet aggregation, thromboxane A_2 generation and antiaggregatory potency of prostacyclin. *Prostaglandins* 1977; 14: 1033-42.
- Zucker MB. Platelet agglutination and vasoconstriction as factors in spontaneous hemostasis in normal, thrombocytopenic, heparinized and hypothyrombinemic rats. *Am J Physiol* 1947; 148: 275-88.
- Zucker MB, Borrelli J. Platelet clumping produced by connective tissue suspensions and by collagen. *Proc Soc Exp Biol Med* 1962; 109: 779-87.
- Zucker MB, Peterson J. Serotonin, platelet factor 3 activity, and platelet-aggregating agent released by adenosine diphosphate. *Blood* 1967; 30: 556.
- Zucker MB, Peterson J. Inhibition of adenosine diphosphate-induced secondary aggregation and other platelet functions by acetylsalicylic acid ingestion. *Proc Soc Exp Biol Med* 1968; 127: 547-51.

